

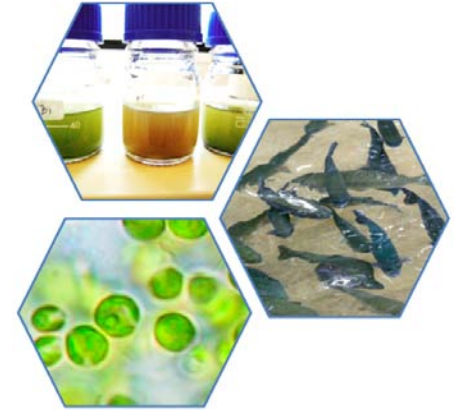
# Comparison of Different Biological Processes for End-of-Pipe Treatment of Recirculating Aquaculture System Effluent

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January 2013



## OUTLINE



- 1. INTRODUCTION TO THE PROBLEM
- 2. OBJECTIVES
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- 4. RESULTS AND DISCUSSIONS
- 5. COMPARISON OF THE RESULTS OBTAINED FROM DIFFERENT BIOLOGICAL PROCESSES
- 6. COMPARISON OF THE BEST FOUND TECHNOLOGY COMPARED WITH THE DANISH STATE OF ART (DAMBRUGSBEKENDTGØRELSEN BEK NR. 130 2012/CHAPTER 4).
- 7. CONCLUSIONS



## 1. INTRODUCTION TO THE PROBLEM

- Aquaculture is named to be a feasible solution to provide enough food for satisfying increasing market demand.
- EU want to consolidate its Aquaculture technology under global market by selling know-how.

*(European comission, 2009)*

- In Denmark discharged nutrients coming from commercial farms is a major challenge.
- Not allowing farmers to increase their production capacity and accomplish environmental regulations (Feed quota allowance/treatment discharge efficiency).

*(Dambrugsbekendtgørelsen BEK nr. 130 2012)*



## 1. INTRODUCTION TO THE PROBLEM

- Aquaculture recirculating systems (RAS)
  - ✓ More intensive production systems (30 – 50 Kg/m<sup>3</sup>)
  - ✓ Require less make up water (<5% volume/day)
  - ✓ Higher control over culture conditions, majorly NH<sub>4</sub> and NO<sub>2</sub> removal
- No major control is often applied for treating Nutrient (N and P) and organic matter from RAS effluent.
- For increasing aquaculture productive goals in Denmark and accomplish environmental legislation, major attention has gained End-of-Pipe treatment (MTF).
- The following project evaluated different biological treatment as End-of-Pipe treatment and compared against Model trout farm technology (Feed allowance quota).



## 2. OBJECTIVES

### General Objective

The general objective of this thesis project is to compare microalgal culture, denitrification and anaerobic digestion for treating RAS effluent as End-of-Pipe treatment.

### Specific Objectives

The specific objectives of this thesis project are to:

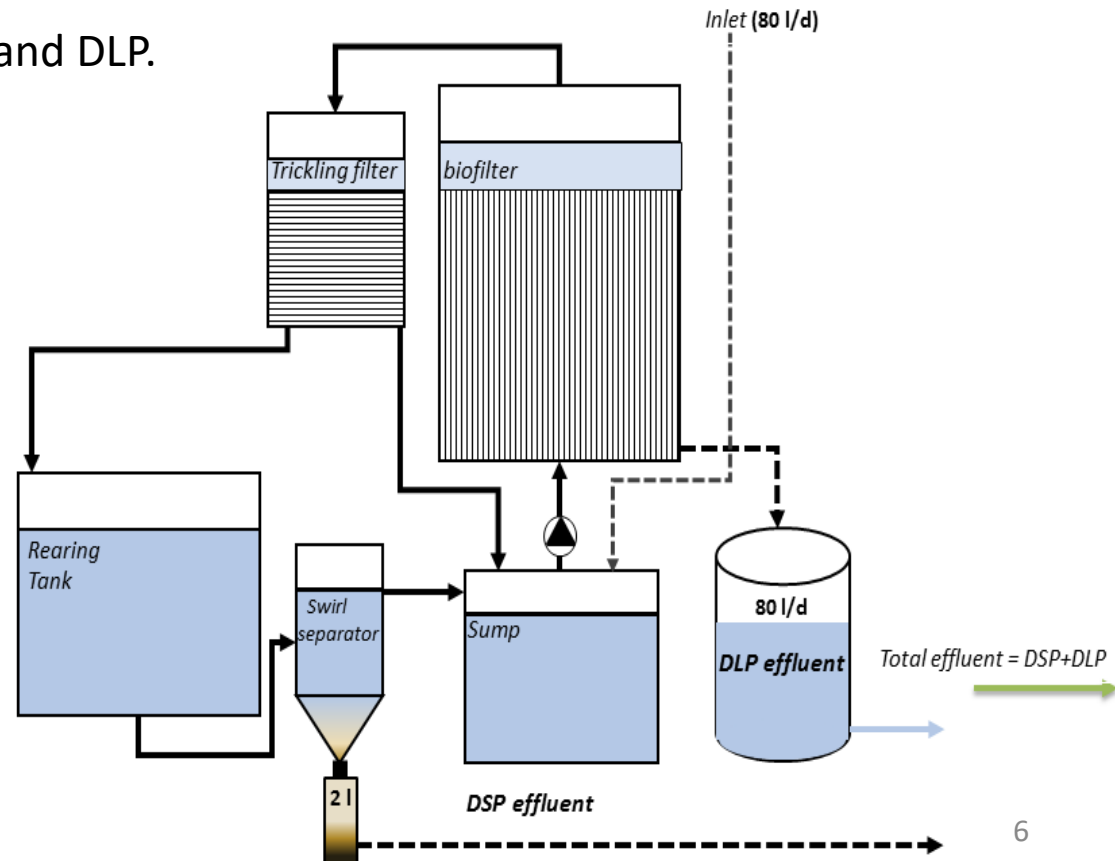
Characterize the composition of RAS effluent for nitrogenous compounds,  $\text{PO}_4\text{-P}$  and organic content (TS, TVS, COD).

Evaluate the removal efficiency of microalgal culture, denitrification and anaerobic digestion for TN, organic content and  $\text{PO}_4\text{-P}$ .

Compare the proposed end of pipe treatment against the actual state of art technology using the feed allowance quota method.

### 3. MATERIAL AND METHODS

- Three different biological processes were evaluated (microalgal treatment, denitrification and anaerobic digestion).
- Effluent coming from 1700 L RAS cultivating rainbow trout (*Onchorynchus mykiss*) (25 Kg) and feeding rate of 300 g pellet/day was used for evaluation.
- The effluent was divided in DSP and DLP.



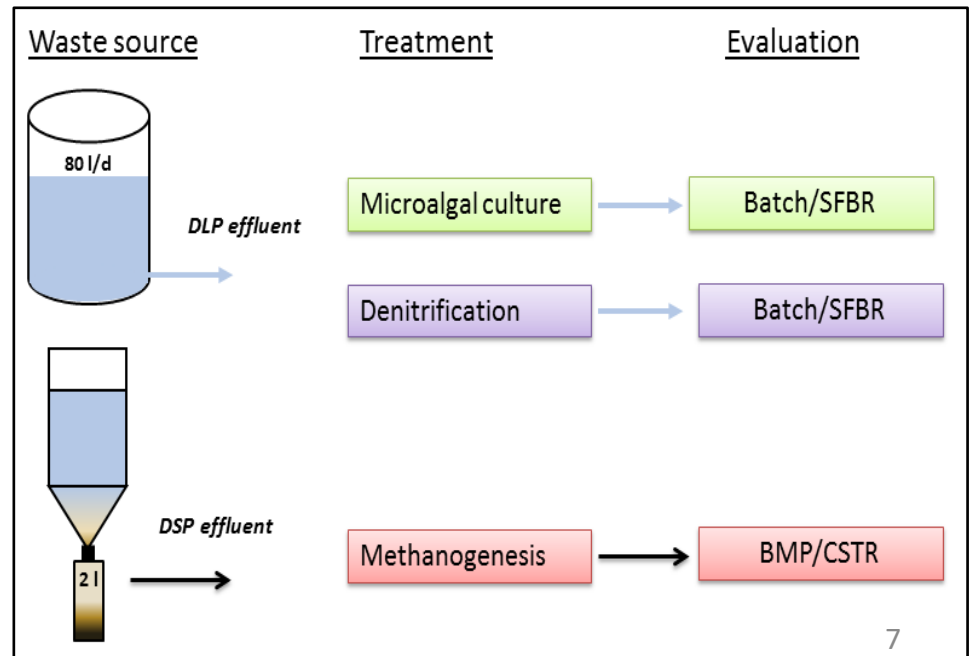
### 3. MATERIAL AND METHODS

#### Characterization of DSP and DLP

- Samples from DSP and DLP were taken daily and frozen for accumulation.
- Each type of waste was analyzed for:
  - Nutrients (N and P):  $\text{NH}_4\text{-N}$ ,  $\text{NO}_3\text{-N}$ ,  $\text{NO}_2\text{-N}$ ,  $\text{PO}_4\text{-P}$  and TKN
  - Organic matter as COD and TVS

#### Removal Efficiencies

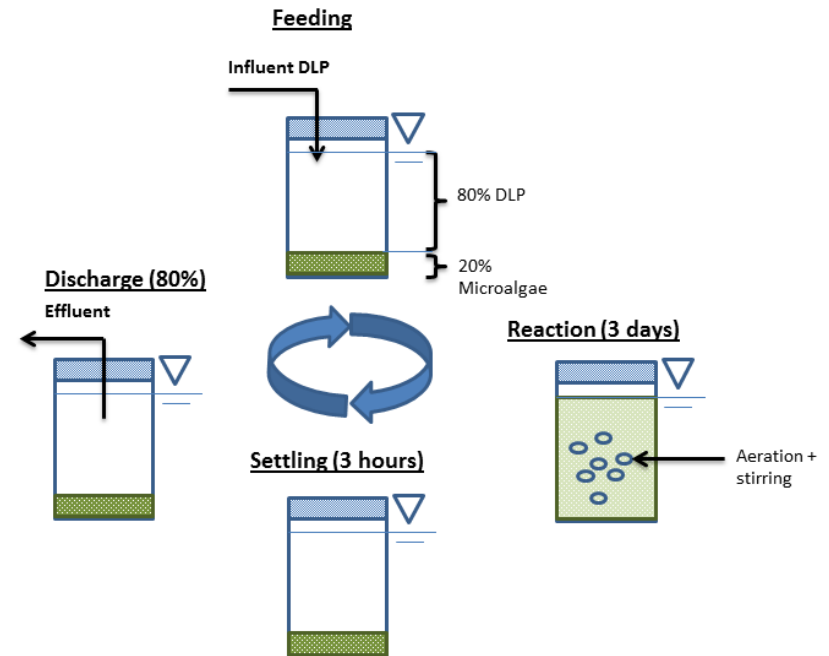
- A series of experiments were performed to evaluate removal efficiencies of TN,  $\text{PO}_4\text{-P}$  and organic content from DSP and DLP.



### 3. MATERIAL AND METHODS

#### Microalgal Treatment for DLP

- Mixotrophic *Chlorella protothecoides* was chosen as microalgal specie.
- Algae was maintained under low light conditions ( $40 \mu\text{E}/\text{m}^2\text{s}^2$ ) with constant air supply.
- Microalgal growth ( $\mu$ ) was related to fluorescence measurements (Em. 445 nm Ex. 670 nm).
- 1 L batch reactors were used for estimating removal  $\eta$  (20% v/v inoculation).
- SFBR were used for long term evaluation under 5 full cycles (20% v/v inoculation).



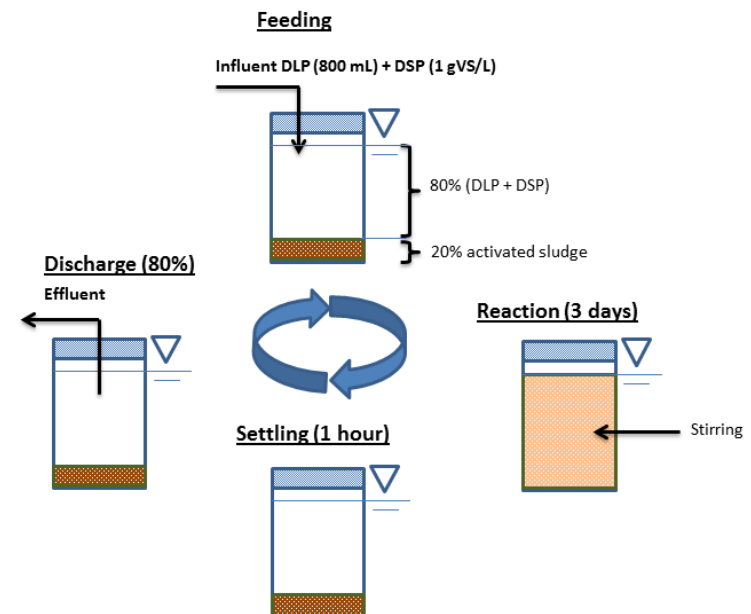


### 3. MATERIAL AND METHODS

#### Denitrification Treatment for DLP

#### + 1 g/L TVS DSP

- Inoculum obtained from activated sludge reactor in the wastewater plant of Lundtofte.
- The medium consisted of 800 mL DLP, 200 mL of inoculum and 25 mL (1 g/L TVS) of DSP in order to supply a fresh organic source.
- Nitrogen was purged to achieve anoxic conditions.
- 1 L batch reactors were used for estimating removal  $\eta$ .
- SFBR were used for long term evaluation under 4 full cycles.



### 3. MATERIAL AND METHODS

#### Anaerobic digestion for DSP

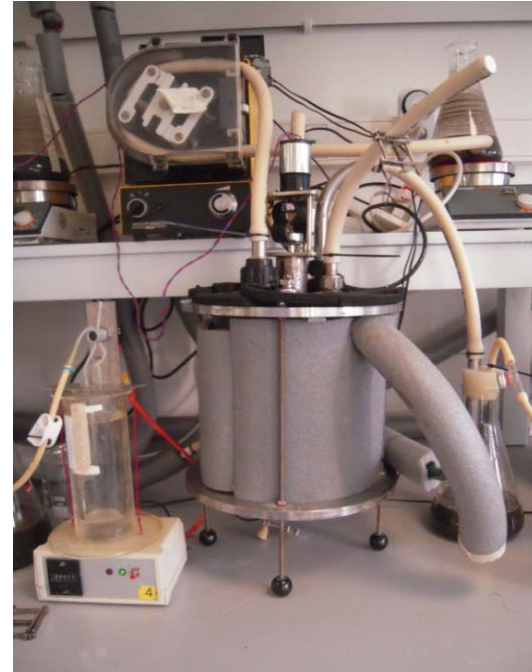
- Biochemical methane potential assay was performed using rubber closed bottles (540 mL).
- Three concentrations of DSP were evaluated 8 g/L TVS (25%), 4 g/L TVS (12.5%) and 2 g/L TVS (6.35%) using digested cow manure as inoculum.
- Avicel was used as control (0.5 g).
- Nitrogen was purged to achieve anaerobic conditions and incubated at 35°C for 21 days.
- Methane content was measured constantly using gas chromatography.



### 3. MATERIAL AND METHODS

#### Anaerobic digestion of DSP

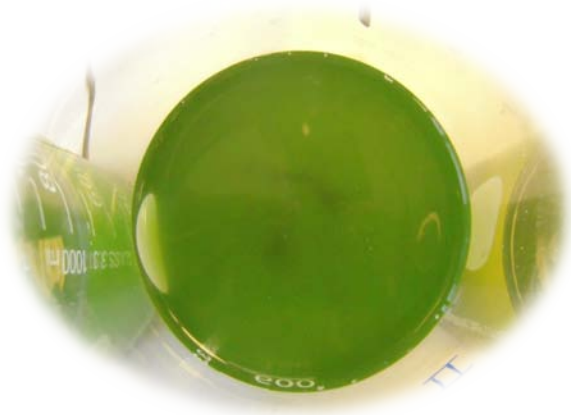
- The treatment of DSP was evaluated under 2 CSTR with reaction volume of 2.5 L.
- HRT of 16 days was used supplying 160 mL/d of substrate.
- For registering steady state conditions recycle effluent from WWTP was used before adding DSP.
- Once obtained steady state conditions reactor 1 was fed with 100% DSP and reactor 2 with 50% previous substrate and 50% DSP.
- Gas analysis using gas cromathograph and pH values were measured constantly to control process status.



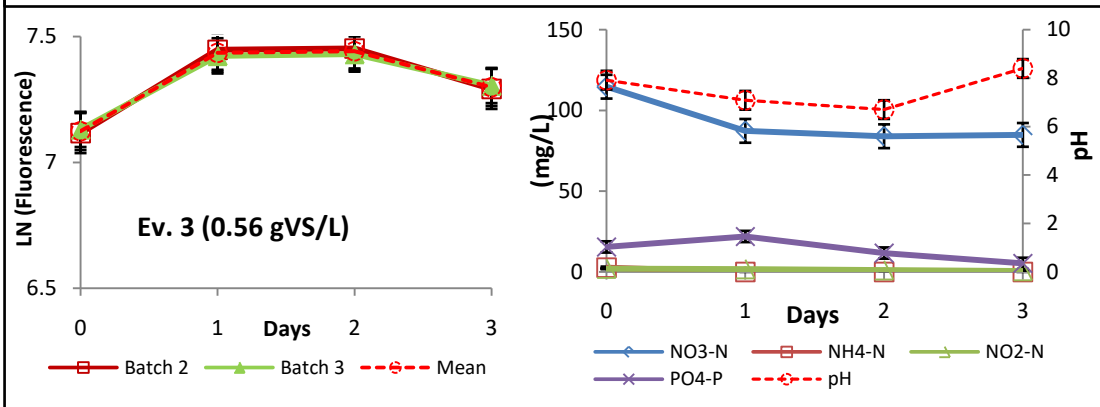
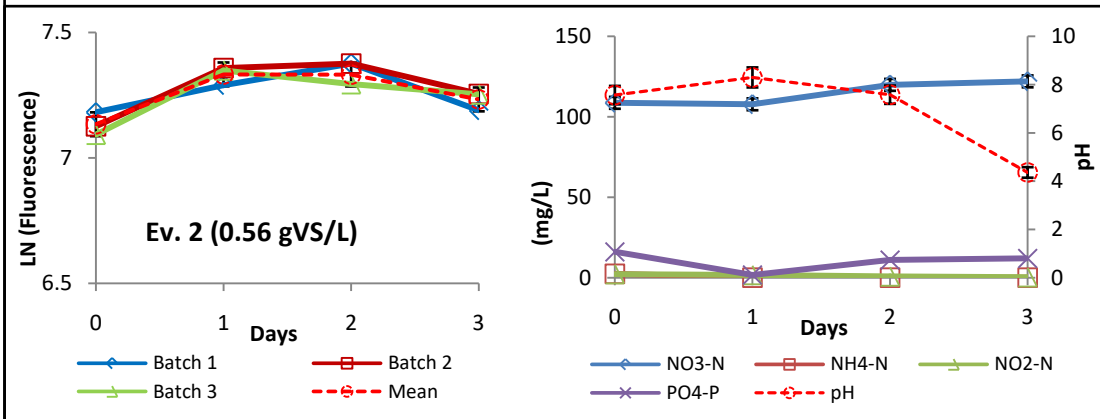
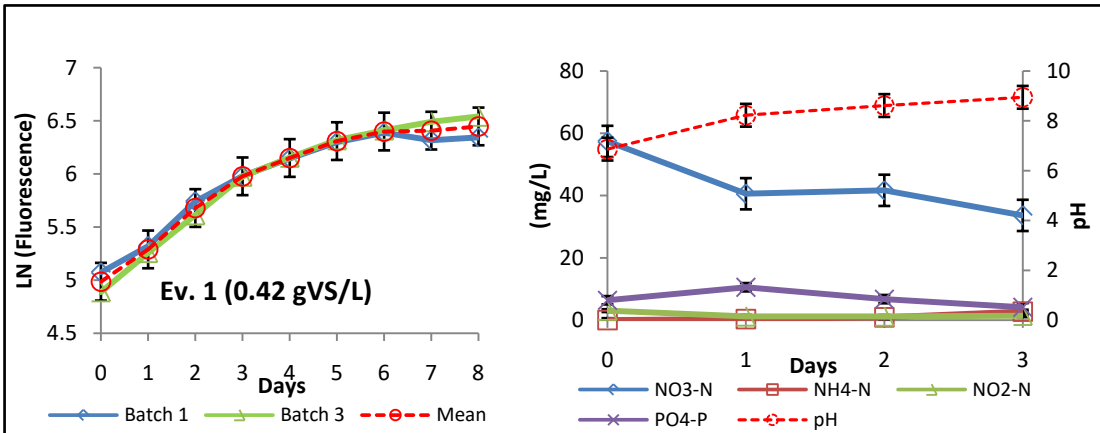


## 4. RESULTS AND DISCUSSION

### MICROALGAL BATCH AND STEP FED BATCH REACTOR (SFBR) EXPERIMENT



# Microalgae Batch Evaluation



# Microalgae Growth

|                     | $\mu d^{-1}$ | Generation Time/day | Division/day | Std. Desv. | CV |
|---------------------|--------------|---------------------|--------------|------------|----|
| <b>Evaluation 1</b> | 0.33         | 2.07                | 0.48         | 0.03       | 8% |
| <b>Evaluation 2</b> | 0.11         | 6.5                 | 0.16         | 0.01       | 9% |
| <b>Evaluation 3</b> | 0.16         | 4.5                 | 0.22         | 0.01       | 7% |

## Evaluation 1

| Concentration (mg/L) | Day 0 | Day 1 | Day 2 | Day 3 | Total Removal |
|----------------------|-------|-------|-------|-------|---------------|
| NO <sub>3</sub> -N   | 59.80 | 24.13 | 33.40 | 26.80 | 55%           |
| NH <sub>4</sub> -N   | 0.05  | 0.43  | 1.20  | 4.02  | 0%            |
| NO <sub>2</sub> -N   | 2.92  | 1.12  | 1.05  | 1.16  | 60%           |
| PO <sub>4</sub> -P   | 6.34  | 9.97  | 7.73  | 5.07  | 20%           |

| TIN (mg/L) | 62.77 | 25.68 | 35.65 | 31.98 | 49% |
|------------|-------|-------|-------|-------|-----|
|------------|-------|-------|-------|-------|-----|

## Evaluation 2

| Concentration (mg/L) | Day 0  | Day 1  | Day 2  | Day 3  | Total removal |
|----------------------|--------|--------|--------|--------|---------------|
| NO <sub>3</sub> -N   | 108.67 | 107.75 | 119.83 | 122.00 | 0%            |
| NH <sub>4</sub> -N   | 2.42   | 0.06   | 0.05   | 0.05   | 98%           |
| NO <sub>2</sub> -N   | 2.20   | 1.69   | 0.78   | 0.30   | 86%           |
| PO <sub>4</sub> -P   | 16.07  | 1.73   | 11.03  | 12.00  | 25%           |

| TIN (mg/L) | 113.28 | 109.50 | 120.65 | 122.32 | 0% |
|------------|--------|--------|--------|--------|----|
|------------|--------|--------|--------|--------|----|

## Evaluation 3

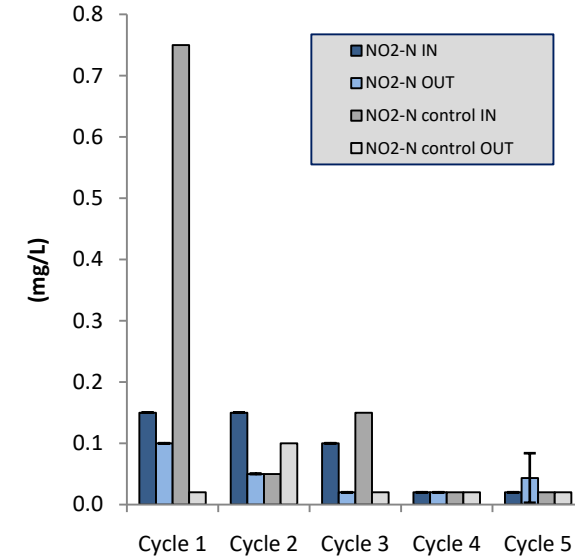
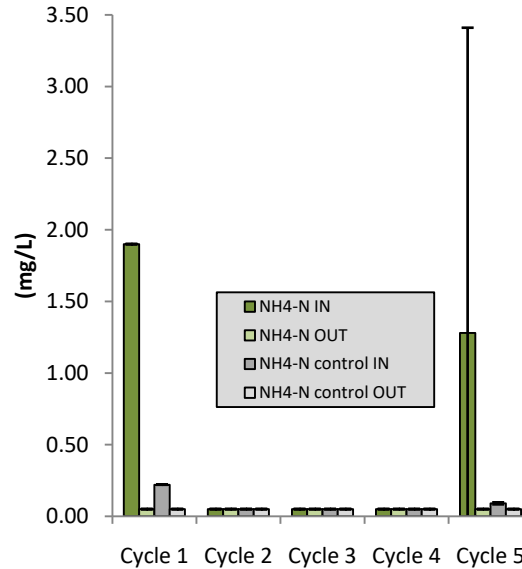
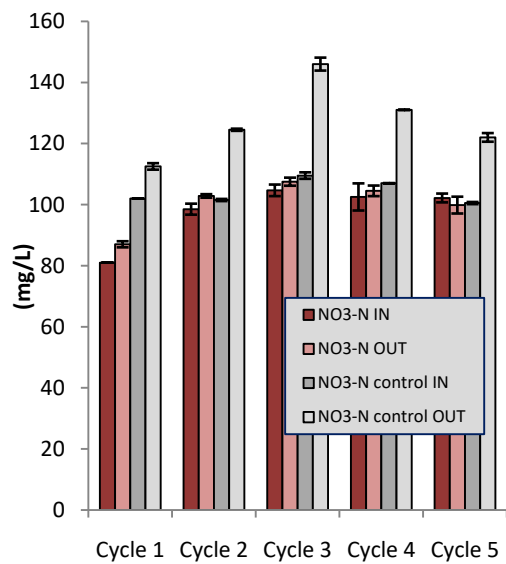
| Concentration (mg/L) | Day 0  | Day 1 | Day 2 | Day 3 | Total removal |
|----------------------|--------|-------|-------|-------|---------------|
| NO <sub>3</sub> -N   | 114.67 | 87.33 | 84.00 | 84.83 | 26%           |
| NH <sub>4</sub> -N   | 2.67   | 0.05  | 0.05  | 0.05  | 99%           |
| NO <sub>2</sub> -N   | 2.08   | 1.78  | 1.13  | 0.62  | 70%           |
| PO <sub>4</sub> -P   | 15.50  | 21.97 | 11.72 | 5.33  | 66%           |

| TIN (mg/L) | 119.42 | 89.15 | 85.18 | 85.45 | 28% |
|------------|--------|-------|-------|-------|-----|
|------------|--------|-------|-------|-------|-----|



# 4. RESULTS AND DISCUSSION

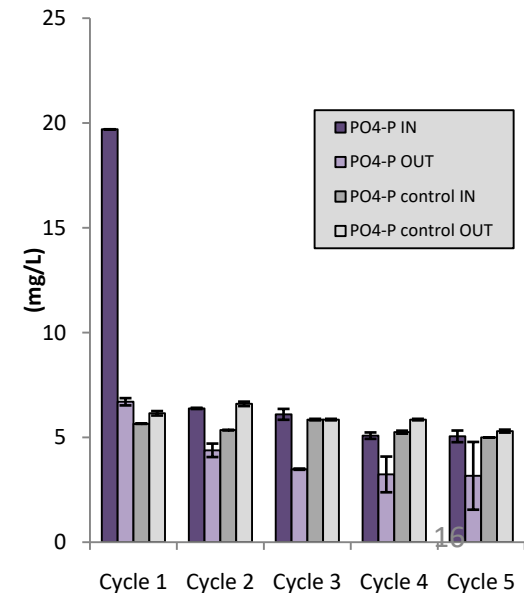
## Microalgal SFBR – Nutrient Removal



|                         | Cycle 1<br>Removal $\eta_1$ | Cycle 2<br>Removal $\eta_2$ | Cycle 3<br>Removal $\eta_3$ | Cycle 4<br>Removal $\eta_4$ | Cycle 5<br>Removal $\eta_5$ |
|-------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| <b>NO<sub>3</sub>-N</b> | 0%                          | 0%                          | 0%                          | 0%                          | 2%                          |
| <b>NH<sub>4</sub>-N</b> | 97%                         | 0%                          | 0%                          | 0%                          | 96%                         |
| <b>NO<sub>2</sub>-N</b> | 33%                         | 58%                         | 90%                         | 0%                          | 0%                          |
| <b>PO<sub>4</sub>-P</b> | 66%                         | 31%                         | 43%                         | 36%                         | 37%                         |
| <b>TIN (mg/L)</b>       | <b>0%</b>                   | <b>0%</b>                   | <b>0%</b>                   | <b>0%</b>                   | <b>3%</b>                   |

| COD in SFBR    | Removal $\eta_1$ | Removal $\eta_2$ | Removal $\eta_3$ | Removal $\eta_4$ | Removal $\eta_5$ |
|----------------|------------------|------------------|------------------|------------------|------------------|
| <b>B1</b>      | 82%              | 89%              | 93%              | 94%              | 77%              |
| <b>B2</b>      | 78%              | 89%              | 91%              | 90%              | 99%              |
| <b>B3</b>      | 81%              | 88%              | 81%              | 92%              | 87%              |
| <b>Control</b> | 94%              | 94%              | 94%              | 94%              | 94%              |
| <b>Mean</b>    | 80%              | 89%              | 88%              | 92%              | 88%              |

COD IN:  
820 mg/L





## 4. RESULTS AND DISCUSSION

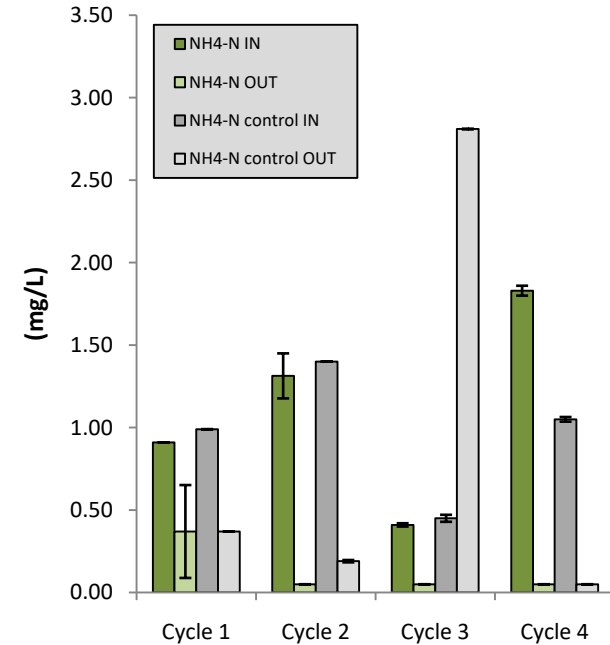
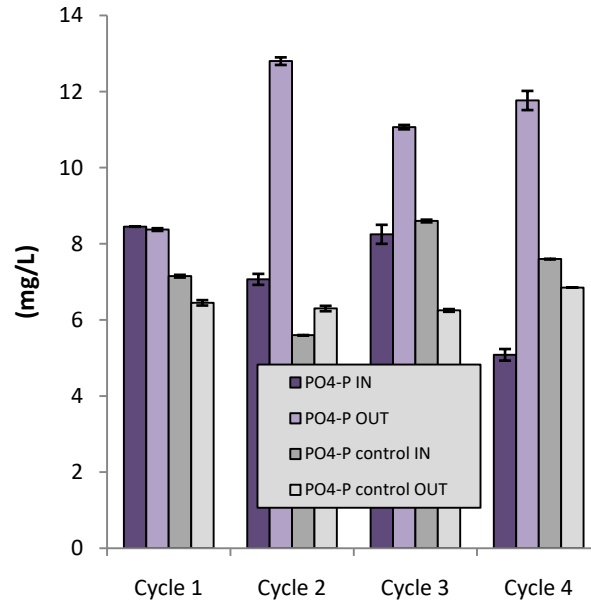
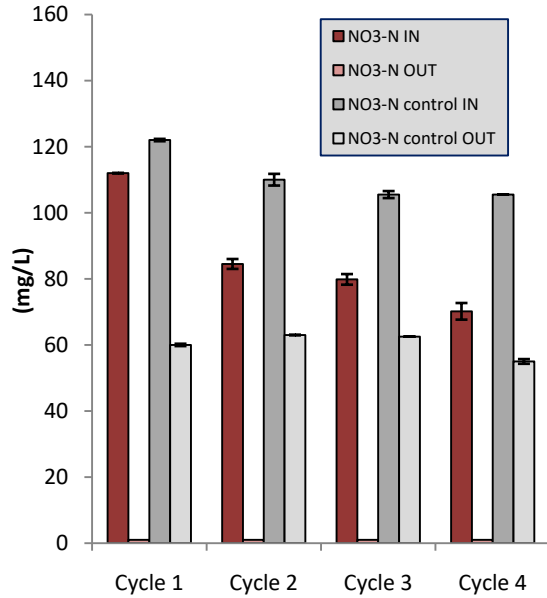
### DENITRIFICATION BATCH AND STEP FED BATCH REACTOR (SFBR) EXPERIMENT





# 4. RESULTS AND DISCUSSION

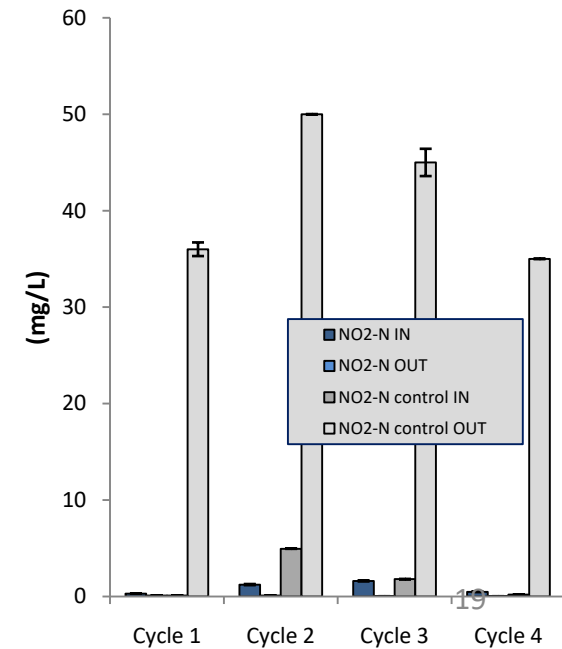
## Denitrification SFBR



|                    | Cycle 1<br>Removal η <sub>1</sub> | Cycle 2<br>Removal η <sub>2</sub> | Cycle 3<br>Removal η <sub>3</sub> | Cycle 4<br>Removal η <sub>4</sub> |
|--------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| NO <sub>3</sub> -N | 99%                               | 99%                               | 99%                               | 99%                               |
| NH <sub>4</sub> -N | 59%                               | 96%                               | 88%                               | 97%                               |
| NO <sub>2</sub> -N | 67%                               | 92%                               | 99%                               | 96%                               |
| PO <sub>4</sub> -P | 1%                                | 0%                                | 0%                                | 0%                                |
| TIN (mg/L)         | 99%                               | 99%                               | 99%                               | 99%                               |

| COD in SFBR | Removal η <sub>1</sub> | Removal η <sub>2</sub> | Removal η <sub>3</sub> | Removal η <sub>4</sub> |
|-------------|------------------------|------------------------|------------------------|------------------------|
| B1          | 78%                    | 88%                    | 90%                    | 88%                    |
| B2          | 88%                    | 86%                    | 88%                    | 92%                    |
| B3          | 90%                    | 90%                    | 90%                    | 91%                    |
| Mean        | 85%                    | 88%                    | 89%                    | 91%                    |
| Control     | 87%                    | 95%                    | 95%                    | 91%                    |

COD IN  
1.27 g/L



## 4. RESULTS AND DISCUSSION

### ANAEROBIC DIGESTION TREATMENT



## 4. RESULTS AND DISCUSSION

### Biochemical methane potential

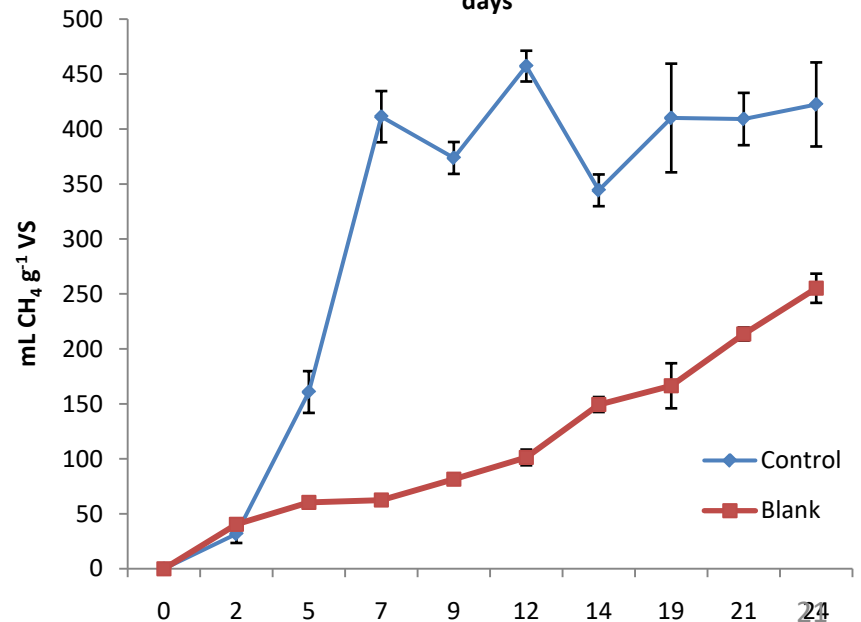
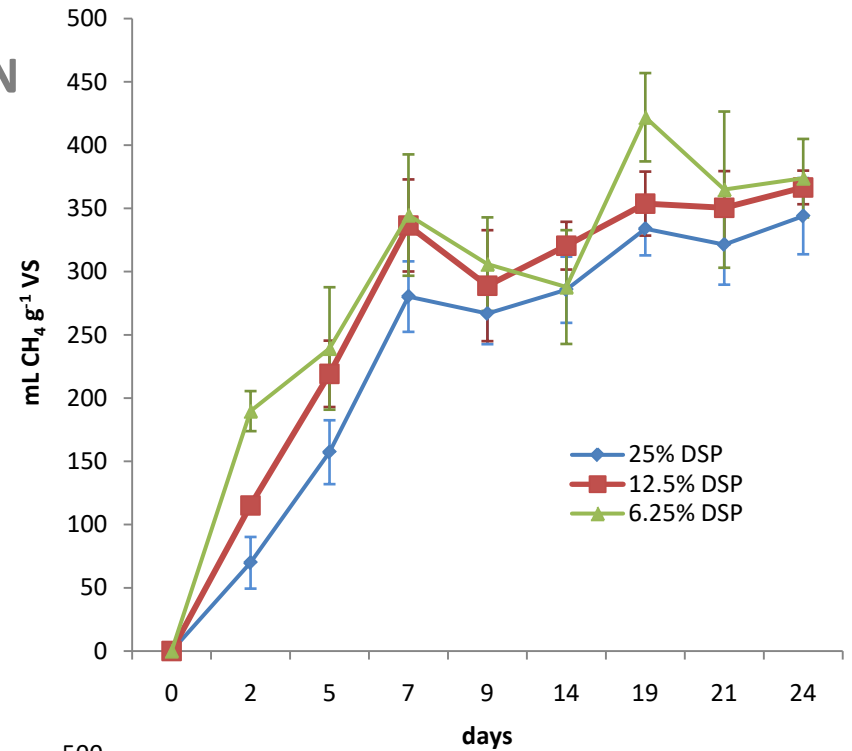
| Energetic balance             | Values |                        |
|-------------------------------|--------|------------------------|
| Fish biomass                  | 25     | Kg                     |
| DSP Volume/d                  | 2.2    | L                      |
| Waste ratio                   | 0.09   | LDSP/Kgfish            |
| CH <sub>4</sub> Yield         | 0.35   | LCH <sub>4</sub> /gTVS |
| SRA CH <sub>4</sub> potential | 14     | LCH <sub>4</sub> /LDSP |
|                               | 0.01   | LCh <sub>4</sub> /kW   |
| Energy production             | 0.14   | kW/L_DSP*d             |
|                               | 0.01   | kW/L_DSP*h             |

|                              |                        |
|------------------------------|------------------------|
| <b>Comercial tank system</b> | 80 MT fish production  |
|                              | 7040 L DSP/d           |
|                              | 98560 LCH <sub>4</sub> |
|                              | 14.4 kW/h              |

### Theoretical nutrient and organic removal efficiencies

- COD removal 60% mesophilic conditions (Gebauer and Eibrokk, 2006).
- 0.23 Kg COD(b)/Kg COD(s) (Henze *et al.*, 2002).
  - ✓ N content in bacteria of 7%
  - ✓ P content in bacteria of 1.4%
- Thus
  - 29.64 gCOD available for bacteria/L DSP
  - 6.8 g/L bacterial mass
  - Requiring 476 mgN/L DSP and 95 mgP/L DSP



## COMPARISON OF TECHNOLOGIES



## 5. COMPARISON OF THE RESULTS OBTAINED FROM DIFFERENT BIOLOGICAL PROCESSES

| DSP                |      |      |        |     | DLP                |      |      |      |     |
|--------------------|------|------|--------|-----|--------------------|------|------|------|-----|
| Concentration      |      |      | Mass   |     | Concentration      |      |      | Mass |     |
| NH <sub>4</sub> -N | 0.23 | mg/L | 0.0003 | g/d | NH <sub>4</sub> -N | 0.24 | mg/L | 0.02 | g/d |
| NO <sub>2</sub> -N | 0.45 | mg/L | 0.001  | g/d | NO <sub>2</sub> -N | 0.75 | mg/L | 0.06 | g/d |
| NO <sub>3</sub> -N | 2.4  | mg/L | 0.006  | g/d | NO <sub>3</sub> -N | 102  | mg/L | 7.9  | g/d |
| PO <sub>4</sub> -N | 25   | mg/L | 0.06   | g/d | PO <sub>4</sub> -N | 5.65 | mg/L | 0.4  | g/d |
| TKN                | 1.21 | g/L  | 2.7    | g/d | TKN                | 0.08 | g/L  | 6.2  | g/d |
| COD                | 49.4 | g/L  | 108.7  | g/d | COD                | 0.82 | g/L  | 63.8 | g/d |
| TN                 | 1.21 | g/L  | 2.7    | g/d | TN                 | 0.18 | g/L  | 14.2 | g/d |

| Technology  | COD (%) | TN (%) | TP (%) | Added value                                    |
|---|---------|--------|--------|--|
| Microalgae<br>( <i>Chlorella protothecoides</i> ) | 33      | 34     | 44     | 0.13 g<br>microalgae/mgNH <sub>4</sub> -N      |
| Denitrification                                   | 96      | 95     | -13    | 3.57 mgCaCO <sub>3</sub> /mgNO <sub>3</sub> -N |
| Anaerobic digestion (biogas)                      | 38      | 9.9    | 11     | 14 LCH <sub>4</sub> /LDSP                      |

## 6. COMPARISON OF THE BEST FOUND TECHNOLOGY WITH THE DANISH STATE OF ART UNDER DAMBRUGSBEKENDTGØRELSEN BEK NR. 130 2012/CHAPTER 4.

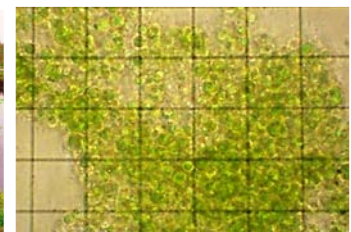
| <b>System</b>                                | <b>TN</b> | <b>TP</b> | <b>BOD</b> | <b>Feed allowance (ton)</b> | <b>Cultivable Ton Fish</b> |
|--|-----------|-----------|------------|-----------------------------|----------------------------|
| <b>Traditional</b>                           | 7%        | 20%       | 20%        | 100                         | 143                        |
| <b>Type 3</b>                                | 15%       | 65%       | 80%        | 109                         | 156                        |
| <b>Type 3 (Wetland)</b>                      | 50%       | 76%       | 93%        | 186                         | 266                        |
| <b>Proposed technology (denitrification)</b> | 95%       | -13%      | 96%(*)     | 1860                        | 2660                       |

(\*)value reported as COD



## 7. CONCLUSIONS

- Source segregation into DSP and DLP effluent fractions from the RAS facilitate the selection of the technology to be applied, due to different masses, nutrients and organic distribution.
- Denitrification showed to be the best option for treating RAS wastewater although phosphorous removal is the major limiting condition for the application of this technology (additional treatment must be considered for its removal).
- The DSP had a higher organic content making it a good substrate for anaerobic digestion. The main drawback of the technology was its low nutrient removal, additional treatment must be considered if anaerobic digestion is chosen for the production of energy in a RAS.
- *Chlorella protothecoides* did not favor growth if  $\text{NH}_4\text{-N}$  was not present in the medium, and the low or null  $\text{NO}_3\text{-N}$  uptake negatively supported the use of this strain for treating RAS effluent (screening of species capable of removing nitrate is fundamental).
- The incorporation of End-of-pipe treatment biological technologies to RAS allow a more sustainable approach, reducing the nutrient emissions and increasing the allowed production capacity, although further research must be done under commercial conditions for the selected technology.



**THANK YOU.**

