Ugent Aquaculture R&D consortium
12th of june 2008

---

ir. Peter De Schryver

Laboratory for Microbial Ecology and Technology
Faculty of Bioscience Engineering
Ghent University - Belgium

Bio-flocs technology for a sustainable aquaculture production

By

Roselien Crab
Peter De Schryver

1. Bio-flocs technology for a sustainable production

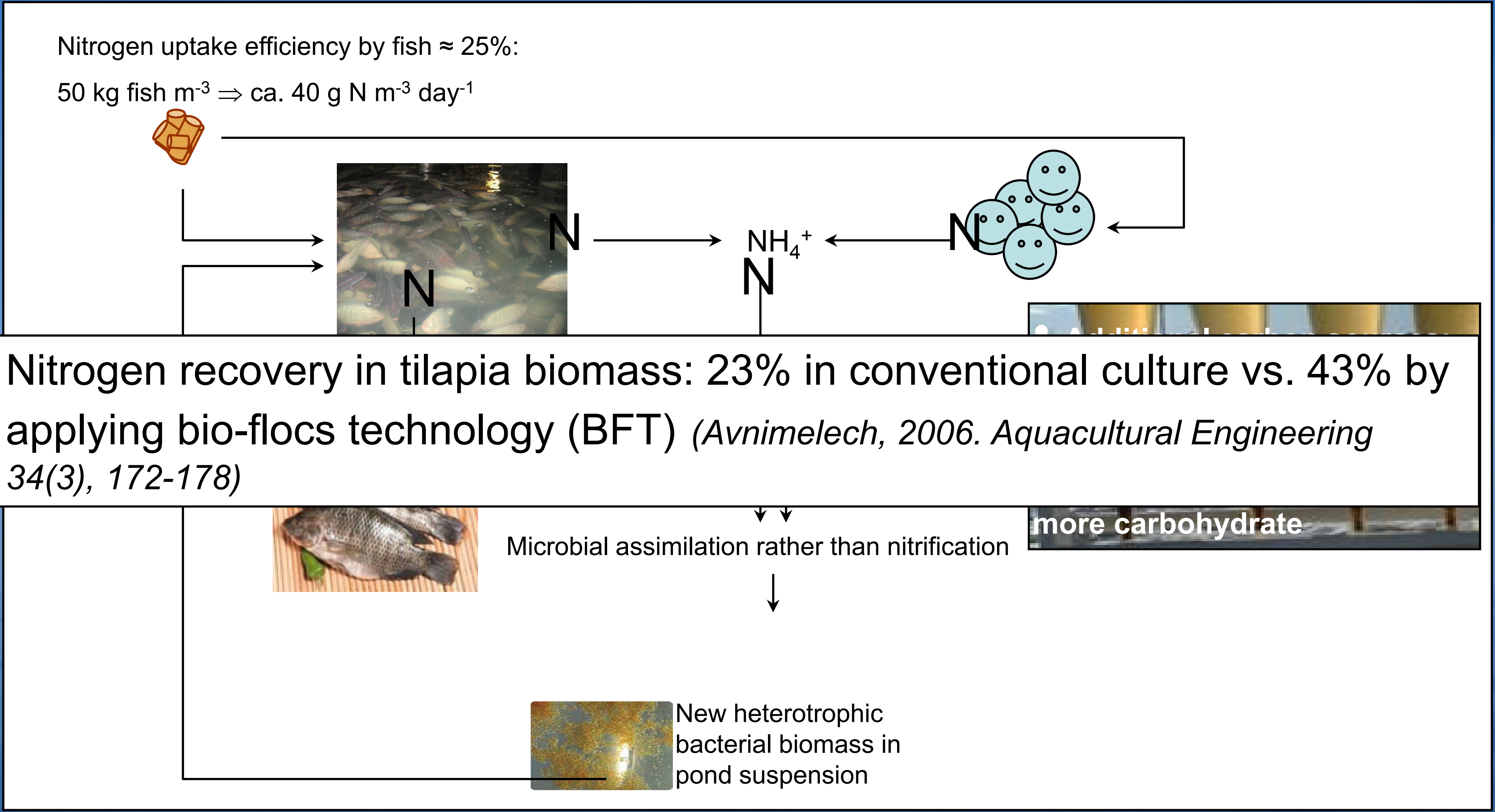
Intensive aquaculture:

- accumulation of compounds in the culture water (ammonia, nitrite, organic matter,...): toxic concentrations and eutrophication in the receiving water bodies *(Piedrahita, 2003. Aquaculture 226(1-4), 35-44)*

- high costs for water treatment in a relatively poor business:
  capital investment costs for
  flow-through systems: 1.3€/kg annual production
  recirculating systems: 5.9€/kg annual production
  (Guttierez-Wing and Malone, 2006. Aquacultural Engineering 34(3), 163-171)

- fishing down the food chain: prime constituents in commercial fish feed (20-30% protein) are fish meal and fishoil
  ⇒ to produce 1 kg fish live weight one needs 1-3 kg feed dry weight
  (Naylor et al., 2000. Nature 405, 1017-1024)

2. The concept of bio-flocs technology



Nitrogen uptake efficiency by fish ≈ 25%:

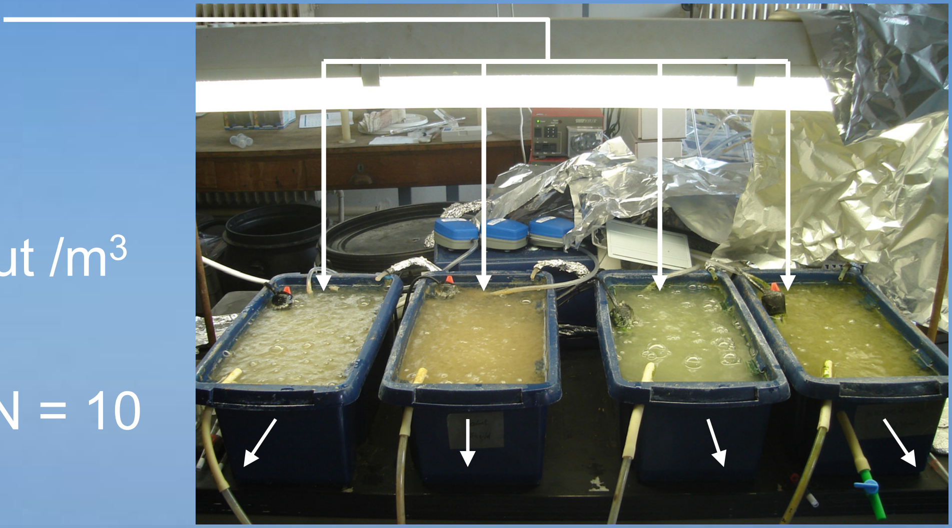
50 kg fish $m^{-3} \Rightarrow$ ca. 40 g N m^{-3} day^{-1}

Nitrogen recovery in tilapia biomass: 23% in conventional culture vs. 43% by applying bio-flocs technology (BFT) *(Avnimelech, 2006. Aquacultural Engineering 34(3), 172-178)*

3. Research on bio-flocs technology - influence of carbon source on system performance

Artificial aquaculture water intensive system (simulated 35 kg fish/m^3)

25 mg TAN/L reactor.day
3,6 mg P/L reactor.day



Aeration: 0,2 kW power input /m^3
HRT: 1 day
Carbon source addition: C/N = 10
at 1 dose/day

Variables:

1) carbon sources: glucose, starch, acetate
2) glycerol + Probiotic inoculum *Bacillus* mixture (INVE) for *Macrobrachium* starvation test

3. Research on bio-flocs technology - influence of carbon source on system performance

Results: water quality

	pH	DO (mg O_2/L)	TAN (mg N/L)	NO_2-N (mg N/L)	NO_3-N (mg N/L)
Glucose	7,4 ± 0,3	7 ± 2	1,3 ± 0,6	0 ± 0	0,05 ± 0,01
Starch	7,0 ± 0,5	6 ± 2	13 ± 1	0,12 ± 0,01	0,58 ± 0,04
Acetate	8,0 ± 0,4	7 ± 1	1,0 ± 0,7	0 ± 0	0 ± 0

	NH_3-N (mg N/L)	NH_4^+-N (mg N/L)
Glucose	0,017 ± 0,008	13 ± 0,6
Starch	0,069 ± 0,005	13 ± 1
Acetate	0,05 ± 0,04	0,9 ± 0,7

- Unionized ammonia-N
  less than 0,05 mg/L
- Nitrite-N
  less than 0,5 mg/L
- Nitrate-N
  toxic at very high concentrations, over 300 mg/L

3. Research on bio-flocs technology - influence of carbon source on system performance

Results: bio-flocs composition

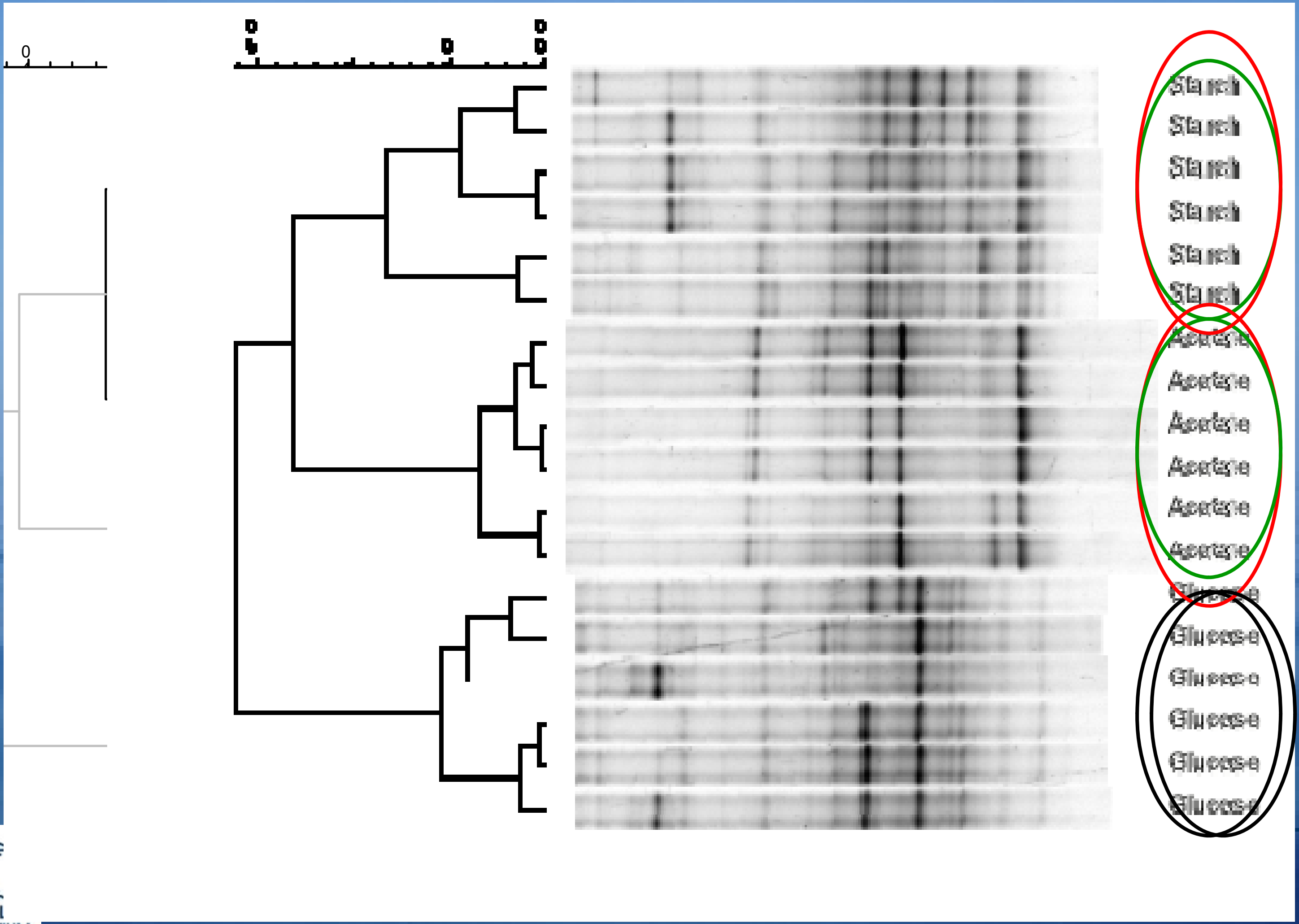
	Fish feed	Glucose	Starch	Acetate
Crude protein	20 - 50	40 ± 6	21 ± 3	19 ± 8
Crude fat	10 - 25	50	17 ± 1	21 ± 11
Ash	< 8.5	5 ± 2	3 ± 2	35
Carbohydrate	15 - 20	5	59	25

	Glucose	Starch	Acetate
18:2(n-6) (mg/g DW)	0.5 ± 0.3	0.7 ± 0.2	0.4 ± 0.2
18:3(n-3) (mg/g DW)	0.050 ± 0.006	0.04 ± 0.03	0.06 ± 0.03
20:5(n-3) (mg/g DW)	0.5 ± 0.1	0.15 ± 0.02	0.08 ± 0.03
22:6(n-3) (mg/g DW)	0.04 ± 0.01	/	/
sum n-6	1.0 ± 0.3	1.0 ± 0.1	0.6 ± 0.1
sum n-3	0.80 ± 0.03	0.30 ± 0.07	0.19 ± 0.08

	Glucose	Starch	Acetate
Vit C (µg/g DW)	0	0	0 - 6

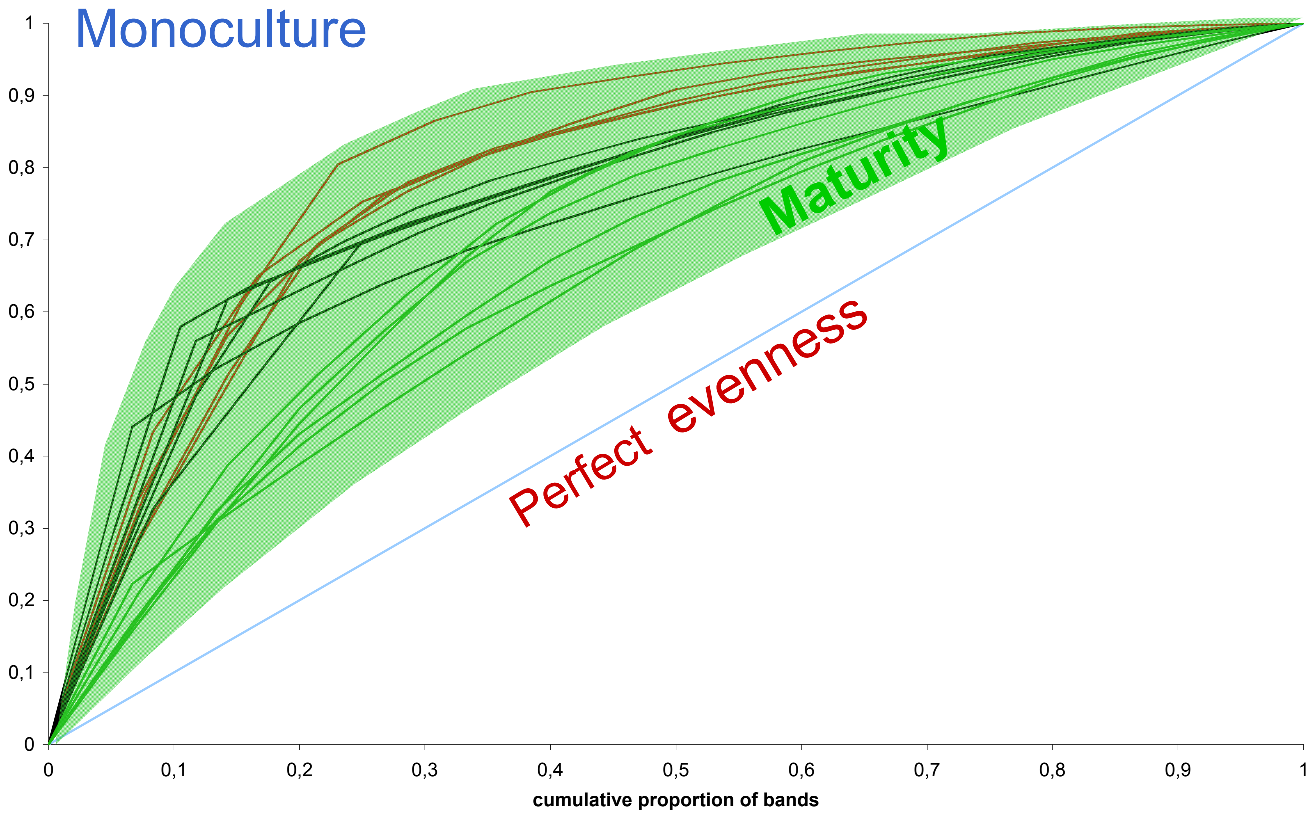
3. Research on bio-flocs technology - influence of carbon source on system performance

Results: bio-flocs composition eukaryotes prokaryotes



3. Research on bio-flocs technology - influence of carbon source on system performance

bacteria



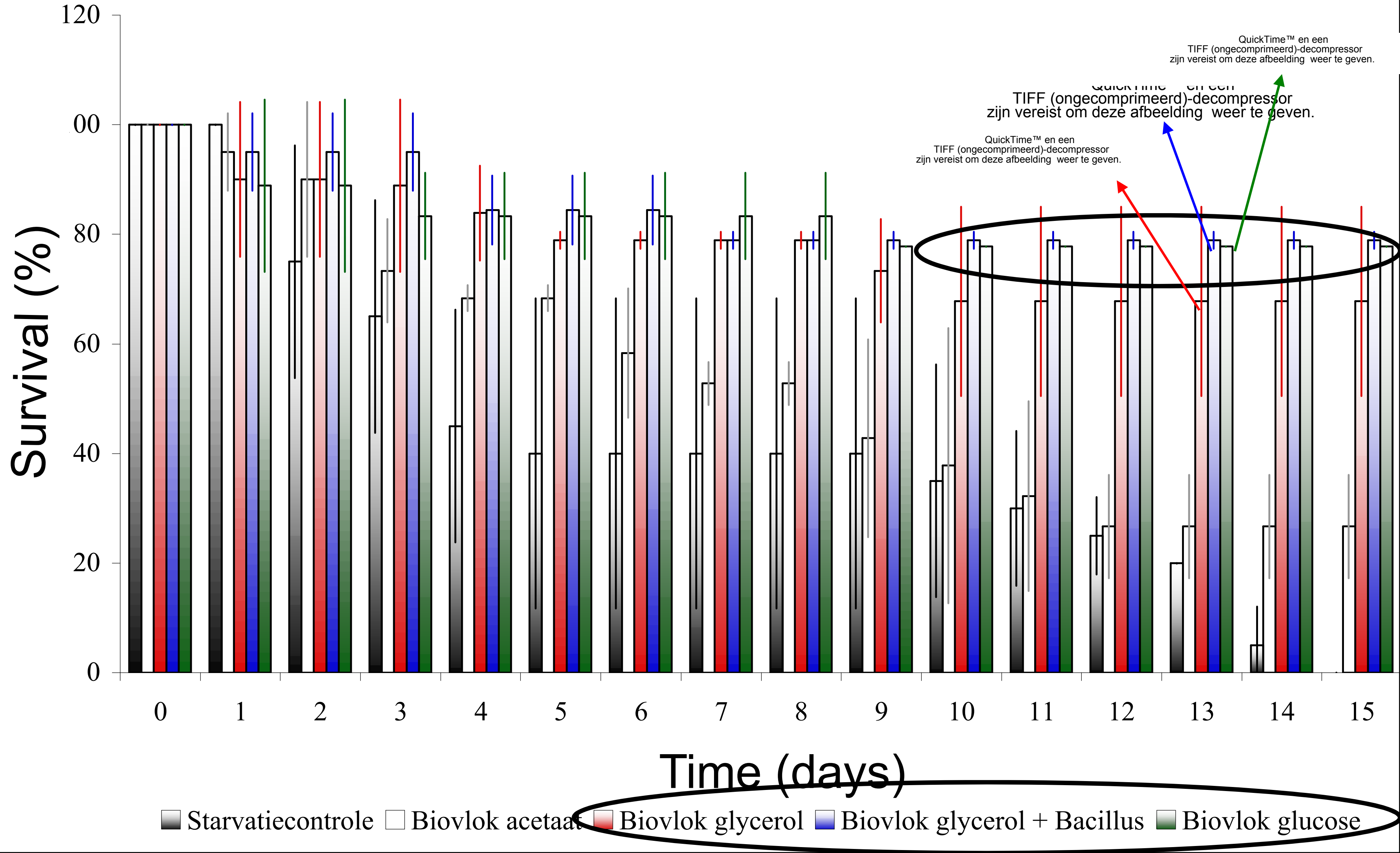
Red: Acetate Black: Glucose Green: Starch

4. Research on bio-flocs technology - *Macrobrachium* starvation test

- Carbon sources tested:
  - * Acetate
  - * Glycerol
  - * Glycerol + initial inoculation of reactors with probiotic inoculum *Bacillus* mixture (INVE)
  - * Glucose

- *Macrobrachium rosenbergii* postlarvae
  - * 10 larvae per treatment, 2 repetitions per treatment
  - * Amount fed based on equal protein addition to all larvae
  - * Survival measured daily

4. Research on bio-flocs technology - *Macrobrachium* starvation test



4. Research on bio-flocs technology - *Macrobrachium* starvation test

	Glucose	Acetate	Glycerol	Glycerol + Bacillus
Crude protein	40 ± 6	19 ± 8	43 ± 1	55 ± 13
Crude fat	50	21 ± 11	3 ± 1	4
sum n-3	0.80 ± 0.03	0.19 ± 0.08	0.6 ± 0	0.7 ± 0.1

Glycerol **⇒ Good feed for maintenance *Macrobrachium***
⇒ Highest nutritional properties
⇒ Cheap carbon source (0.150 €/kg)
⇒ + Bacillus mixture: increase nutritional value

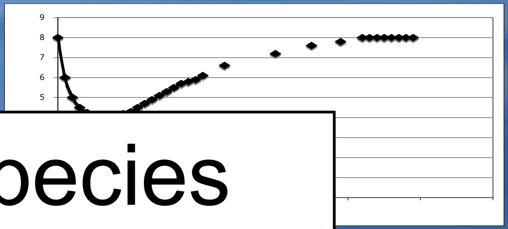
	Glucose	Acetate	Glycerol	Glycerol + Bacillus
Vit C (µg/g DW)	0	0 - 6	6 - 54	8 - 54

5. Research on bio-flocs technology - Sequential Batch Reactor as alternative approach

Many advantages related to bio-flocs technology however:

⇒ high turbidity of the water due to intense microbial growth

⇒ variation in oxygen level due to carbon dosing



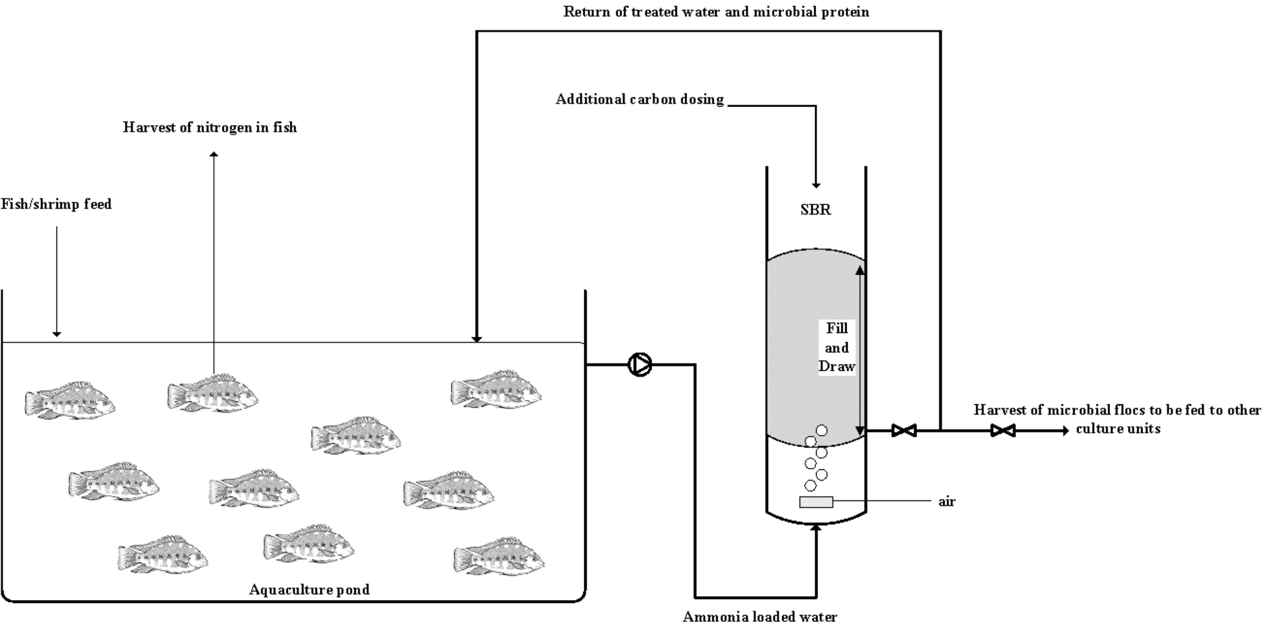
⇒ problems for sensitive species

5. Research on bio-flocs technology - Sequential Batch Reactor as alternative approach

--Artificial aquaculture water intensive system (simulated 23 kg fish/m^3) at 112 mg TAN/L reactor.day

- Aeration: 2.6 kW power input /m^3
- Assessment of different C/N ratios (2.5 - 5 - 10 - 15)

2 SBR's: - glycerol as carbon source
- acetate as carbon source



5. Research on bio-flocs technology - Sequential Batch Reactor as alternative approach

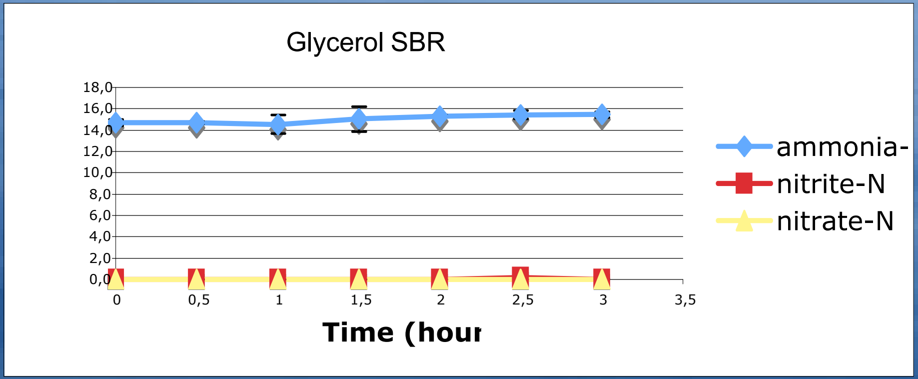
Glycerol SBR

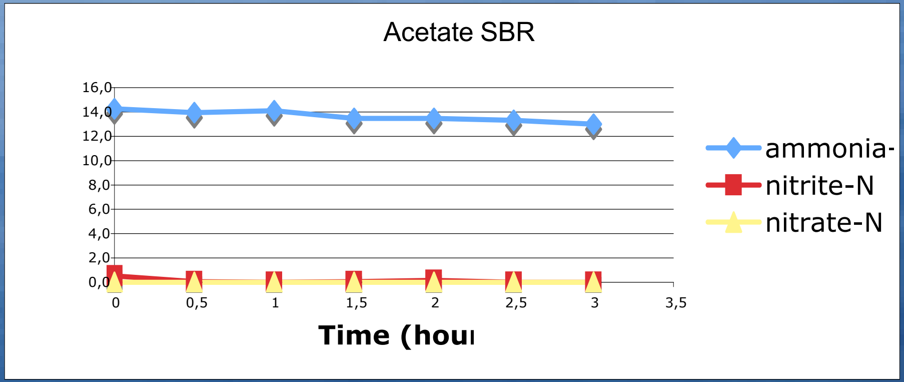
QuickTime™ een
TIFF (ongecomprimeerd) ecompressor
zijn vereist om deze afbeeldin weer te geven.

Acetate SBR

Low values for nitrite and nitrate

Batch nitrification test (C/N 15)





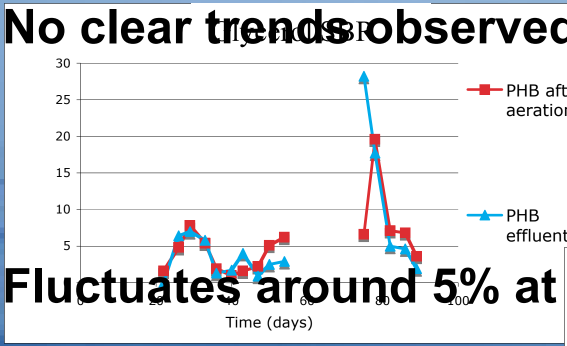
5. Research on bio-flocs technology - Sequential Batch Reactor as alternative approach

Protein content

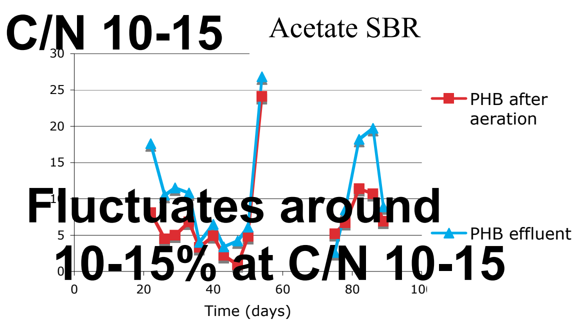
Period (days)	C/N ratio	Effluent Glycerol SBR	Reactor Glycerol SBR	Effluent Acetate SBR	Reactor Acetate SBR
0 Š 11	2,5	3,4 ± 2,8	35,6 ± 13,0	6,7 ± 3,3	23,3 ± 6,4
11 Š 32	5	20,2 ± 20,6	45,0 ± 17,5	28,8 ± 24,1	20,0 ± 12,9
32 Š 50	10	57,6 ± 22,2	60,4 ± 11,0	57,2 ± 20,5	54,4 ± 10,6
50 - 96	15	61,1 ± 12,1	56,8 ± 13,8	43,9 ± 8,6	60,5 ± 16,3

PHB content

No clear trends observed



Fluctuates around 5% at C/N 10-15



Fluctuates around 10-15% at C/N 10-15

The beneficial effect of poly-β-hydroxybutyrate (PHB) and its derivates for aquaculture purposes

by

Peter De Schryver

5. Research on poly-β-hydroxybutyrate - the potential of PHB for aquaculture purposes

Use of short chain fatty acids as biocontrol agents:

⇒ Bacteriostatic and bacteriocidal effects on pathogenic bacteria *(Ricke, 2003. Poultry Science 82, 632-639)*

⇒ Influence invasion capacity of *Salmonella enteridis* in epithelial cells *(Van Immerseel et al., 2003. International Journal of Food Microbiology 85(3), 237-248)*

More specific, use of butyric acid:

⇒ Main energy source for intestinal epithelial cells *(Biagi et al., 2007. Journal of Animal Science 85(4), 1184-1191)*

⇒ Protects *Artemia franciscana* against infection with pathogenic *Vibrio campbellii* *(Defoirdt et al., 2006. Aquaculture 261, 804-808)*

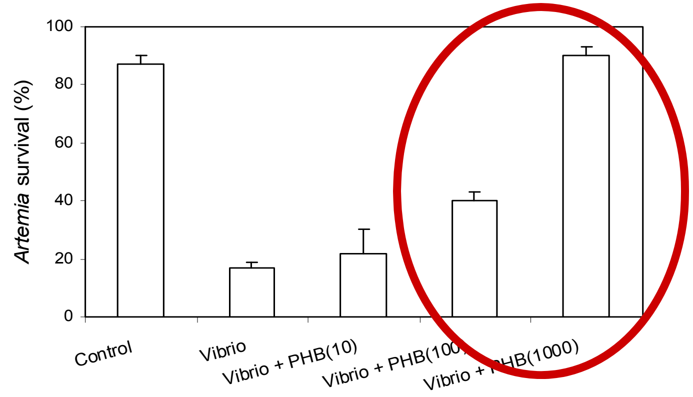
5. Research on poly-β-hydroxybutyrate - the potential of PHB for aquaculture purposes

Supplementation of butyric acid to the aquaculture pond water ⇒ requires high doses due to inefficient uptake from the water by the culture organisms

Possible solution: the bacterial storage compound poly-β-hydroxybutyrate (PHB) = polymer of β-hydroxy butyric acid (3-HB)

5. Research on poly-β-hydroxybutyrate - the potential of PHB for aquaculture purposes

⇒ Feeding with crystalline PHB results in higher survival and in the protection of *Artemia franciscana* from pathogenic *Vibrio campbellii* *(Defoirdt et al., 2007. Environmental Microbiology 9, 445-452)*



Significantly increased survival at 100 mg/l PHB or more

⇒ Effects of supplementing crystalline PHB to the feed of European sea bass juveniles?

5. Research on poly-β-hydroxybutyrate - supplementation of crystalline PHB to sea bass juveniles feed

Sea bass survival

% survival after 6 Weeks

Ph
we
en
re
ne

e

TIFF (
zijn vereist

QuickTime™ en een
TIFF (ongecomprimeerd)-decompressor
zijn vereist om deze afbeelding weer te geven.

⇩

Ef

5. Research on poly-β-hydroxybutyrate - supplementation of crystalline PHB to sea bass juveniles feed

pH of sea bass intestine

QuickTime™ en een
TIFF (ongecomprimeerd)-decompressor
zijn vereist om deze afbeelding weer te geven.

→ Higher uptake of PHB results in lower intestinal pH

⇩

Production of 3-HB in the gut?

To be performed:

- 3-HB production in the gut (bacterial/enzymatic activity)?
- sea bass epithelial cell morphology (SEM)

5. Research on poly-β-hydroxybutyrate - supplementation of bacterial PHB to sea bass juveniles feed

Remark: crystalline PHB = PHB extracted from bacterial cells

⇒ supplementation of PHB containing bacterial cells in feed would be easier

⇒ bacteria loaded with PHB protect *Artemia franciscana* from pathogenic *Vibrio campbellii*

(Halet et al., 2007. FEMS Microbiology Ecology 60(3), 363-369)

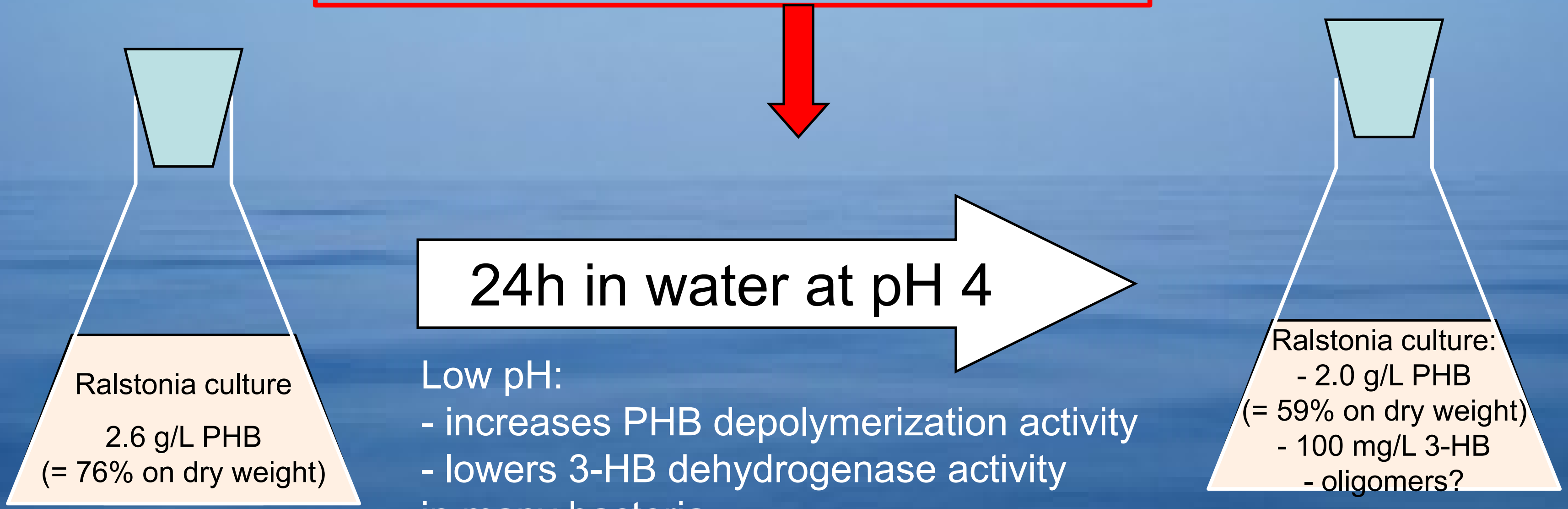
⇒ future experiment: effects of supplementing

bacteria loaded with PHB to the feed of European sea bass juveniles?

5. Research on poly-β-hydroxybutyrate - supplementation of bacterial PHB to sea bass juveniles feed

Experiment variables:

- Feed
- Feed + PHB bacteria
- Feed + PHB bacteria + 3-HB produced by bacteria
- Feed + 3-HB produced by bacteria



Ralstonia culture

2.6 g/L PHB
(= 76% on dry weight)

24h in water at pH 4

Low pH:
- increases PHB depolymerization activity
- lowers 3-HB dehydrogenase activity
in many bacteria

Ralstonia culture:
- 2.0 g/L PHB
(= 59% on dry weight)
- 100 mg/L 3-HB
- oligomers?

5. Research on poly-β-hydroxybutyrate - supplementation of bacterial PHB to sea bass juveniles feed

Hypothesis:

Feed supplemented with alkaline treated PHB containing culture results in triple fold action:

- 3-HB: readily absorbable by stomach and intestine
- Oligomers (?): difference in degradability and thus different availability for the fish
- PHB: slowly biodegradable and thus slow release

⇒ difference in effects compared to crystalline PHB?

Thank you for your attention!!!