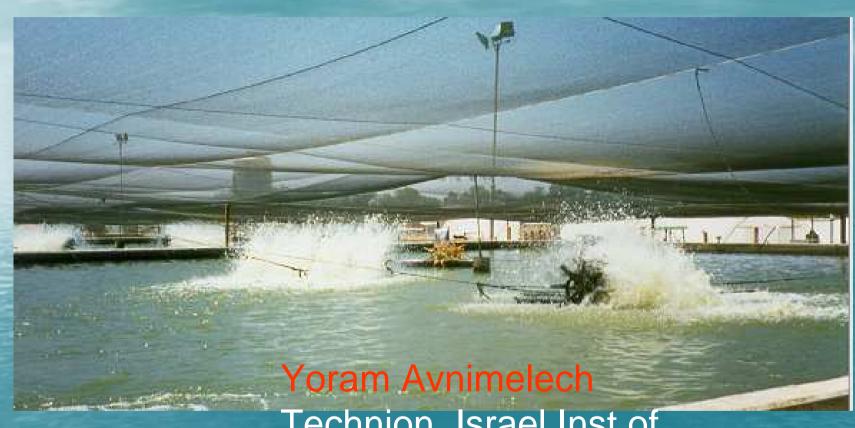
### MICROBIAL CONTROLLED PONDS – PRINCIPLES, IMPLEMENTATION AND NEW DEVELOPMENTS



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ABC of Yoram Avnimelech agyoram@tx.technion.ac.il Microbial Controlled Ponds (MCP):

#### What is common to all MCP's

- 1. Zero or limited water exchange.
- 2. Intensive culture.
- ( $\sim > 10$ kg/m<sup>2</sup> for fish, > 1kg/m<sup>2</sup> for shrimp. possible exception, to be dealt with separately, extensive MCP's)
- 3. Thus: Accumulation of organic residues

(in the range of 100's mgC/l).

4. Thus: dense microbial population (In the range of 10<sup>7</sup> up to possibly 10<sup>9</sup> CFU/ml)



## **Operational Conclusions: Bio-technological controls**

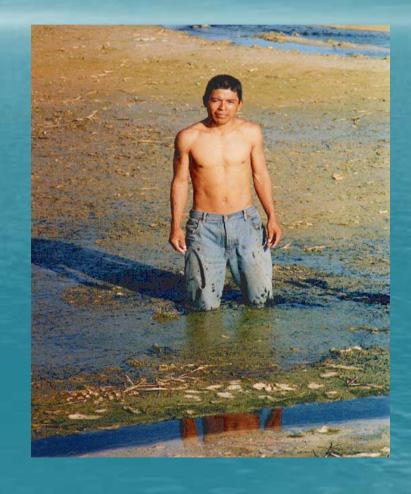


### 1. Prevention of Anaerobic Conditions

- Anaerobic, or anoxic, microbial metabolism leads to the production of undesired compounds like sulfides or organic acids that are toxic to fish and shrimp.
   These compounds may also block nitrification, often leading to accumulation of nitrite.
- Bottom accumulation of sludge leads to development of anaerobic conditions, all over or in specific sites in the pond.

<b>Electron Acceptor</b>	Process	Approximate: Redox
(Oxidizing system)		Potential (mV)
Oxygen O <sub>2</sub>	Aerobic respiration	500-600
AEROBIC	$(C + O_2 \rightarrow CO_2)$	
Nitrate	Denitrification	300-400
NO <sub>3</sub> -	2NO <sub>3</sub> +3C->	
	$3CO_{2+}N_2$	
Organic	F'ermentation:	< 400
Components	Organic Acids	
Fe <sup>+3</sup> , Mn <sup>+4</sup>	Reduction	200
SO <sub>4</sub> , S	Sulfur	-100
	reduction	
CO <sub>2</sub>	Methane	-200
	fermentation	

Sludge accumulation can be controlled by proper selection, location and operation of aerators, planning the pond so as to minimize sludge coverage and by periodic drainage of the sludge.



#### The nitrogen problem

- Fish use just about 25% of feed nitrogen.
   The rest excreted (and not utilized!!).
- Excretion and microbial mineralization generate ammonium. Ammonia is highly toxic.
- Nitrite is also toxic, especially in fresh water systems.
- Ponds are enriched in N as compared to C. Carbon is emitted as CO2. Nitrogen is left in the pond. Can we revert this feature??

#### ALGAE CONTROL OF NITROGEN

- Carbon Assimilation:

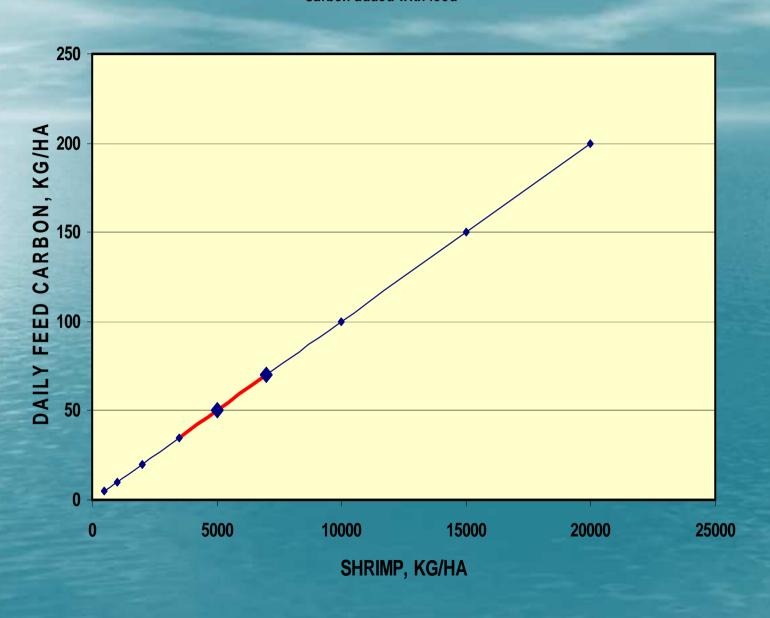
is a production of SUGARS

 However, algae are made mostly of protein, thus they need to take up ammonium from the water.

- \* One Nitrogen
  is needed for
  each 5 Carbon
  assimilated.
- \*Normally,
   assimilation
   capacity is ~
   4g/m² \* day
- \*Thus the algae nitrogen control limit
- Is about 0.8
   g/m² \* day



#### Carbon added with feed





#### MICROBIAL CONVERSION

•MICROBES PRODUCE NEW CELL MATERIAL (protein)
AND ENERGY:

$$\Delta C = CO_2 + \Delta Ccell$$
  
 $\Delta Ccell/\Delta C = \varepsilon$ 

= Microbial conversion efficiency
 = normally, 0.4-0.6 for aerobic microbial processes. Lower for anaerobic.
 Bacteria are rich in respect to N (C:N ~ 4)
 Thus, 1 Nitrogen is taken up for 4 ΔC

#### Manipulating bacteria

- We can add carbon rich and protein poor material (carbo-hydrate, CH), such as starch or cellulose (ground grains, molasses, cassawa etc. To induce accelerated nitrogen uptake.
- Normally, there is more than enough nitrogen for new cell production.



Inorganic nitrogen control is achievable and predictable

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### HOW MUCH CARBON IS agyoram@tx.technion.ac.il MEEDED?

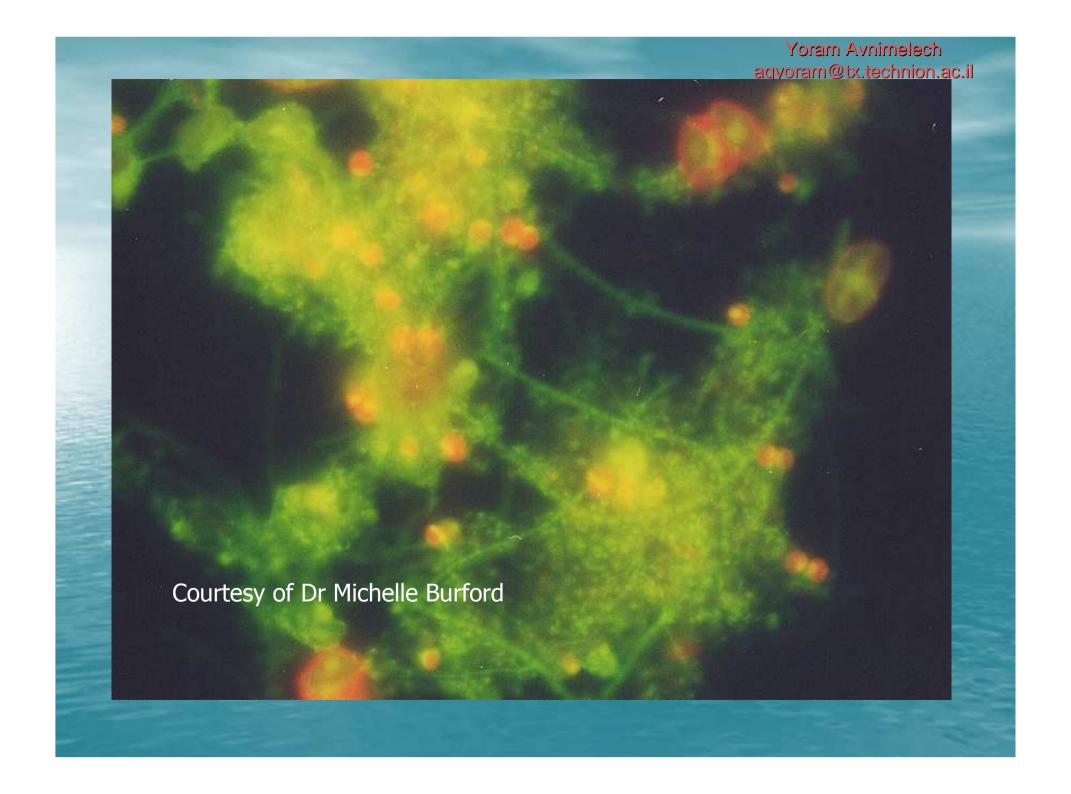
- $\square \triangle N = \triangle Cmic / (C/N)mic = \triangle CH \times \%C \times E/(C/N)mic$
- $(\%C = ca\ 0.5; E = 0.4-0.6; [0.5] / (C/N)mic = 4-$ 6 [4]
- $\square \triangle N = \triangle CH \times 0.05$
- $\Delta CH = 20 \Delta N$
- We have to add 20g carbohydrate (mollases, casawa etc.) to sequester 1 g ammonium nitrogen

#### Feeding fish with bacteria

- We can induce the production of microbial protein.
   Will it be a feed source for fish?
- Can they physically harvest bacteria?
- Is it nutritive?
- Will they digest it?



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#### Protein Recycling

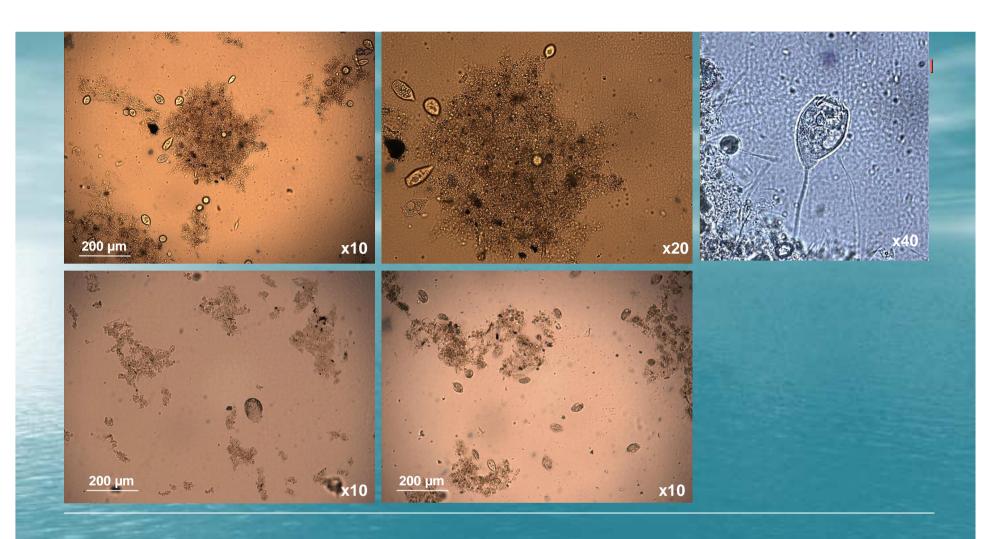
- Normally, fish or shrimp recover just
   ~25% of feed protein.
- In bacterial controlled ponds, they eat the protein twice; Once in the feed and then they consume microbial protein. The protein recovery reaches almost 50%.

Protein is the most expensive part of the feed.

<u>Expt. # 1</u> 51 days	<u>30%</u> <u>Protein</u>	<u>20%</u> <u>Protein</u>
FEED C/N	11.1	<u>16.6</u>
Daily Gain (%)	1.59 <sup>a</sup>	$2.0^{b}$
FCR	2.62	2.17
PCR	4.38	2.42
FEED COST (Kg fish/\$US)	0.848	0.583
Exp. # 2 (30 days)		
<u>C/N</u>	11.1	16.6
Daily gain (%)	1.63°	$2.22^b$
FCR	2.62	2.02
PCR	4.35	2.18
Feed cost (US\$/Kg fish) (1) Protein conversion is protein input in	0.848 n feed / protein gain in fish	0.543

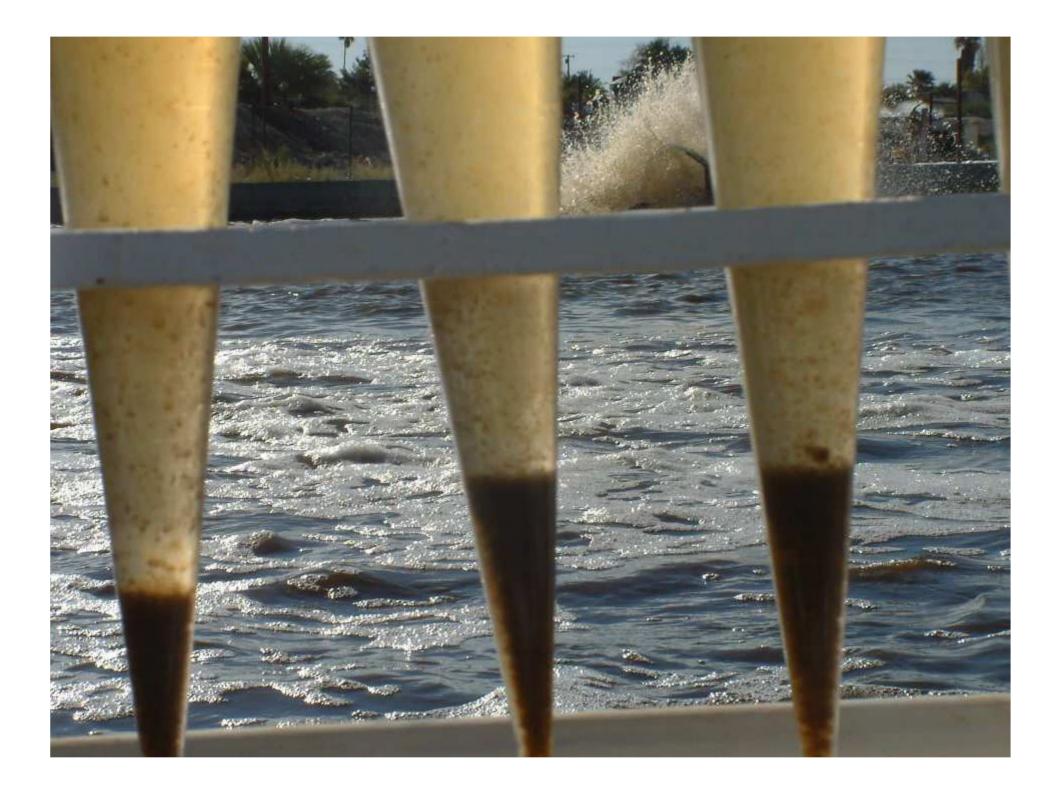
## Pros & Cons of microbial N recycling

- 1. Effective, reliable and predictable inorganic nitrogen control.
- 2. Double protein utilization, thus enables to use cheaper feed: lower protein feed.
- 3. Lower aquaculture dependence on marine fish meal & oil.
- 4 lower bollution
- 5. Slightly higher oxygen consumption.
- 6. High water turbidity, may be a problem to some species.

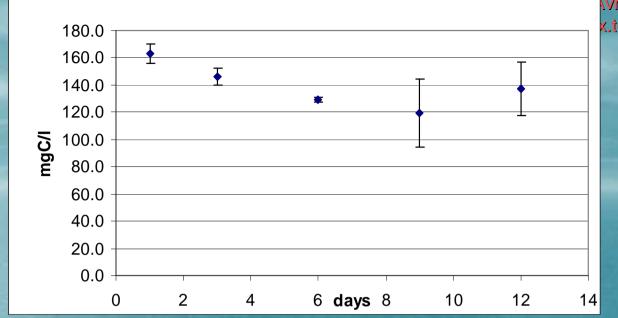


#### Flocs and feeding

O







Changes of suspended carbon with time C = 168 - 6.61 t R2 = 0.986Daily uptake by fish 0.59 g carbon  $Results \ in \ experimental \ tanks,$   $Pacific \ Aquafarms, \ CA$ 

### Uptake of flocs by tilapia as measured using 15N, TSS Floc Volume, Suspended C & N Pacific Aquafarm, CA, 2004 (Avnimelech, GAA Advocate 2005)

	TSS	Floc Volume	Carbon	Nitrogen
Daily measured change	20 mg/l	1.74 ml/l	6.61 mg/l	0.87 mg/l
Equivalent dry SS change (mg/l)	20	24.3	26.9	23.5
Daily uptake by fish as equivalent SS (g/kg fish)	8.92	10.79	11.03	9.66 (2.34 g protein)
Daily uptake of Nitrogen determined through 15N accumulation				415 mg N, 2.68 g protein/ kg fish

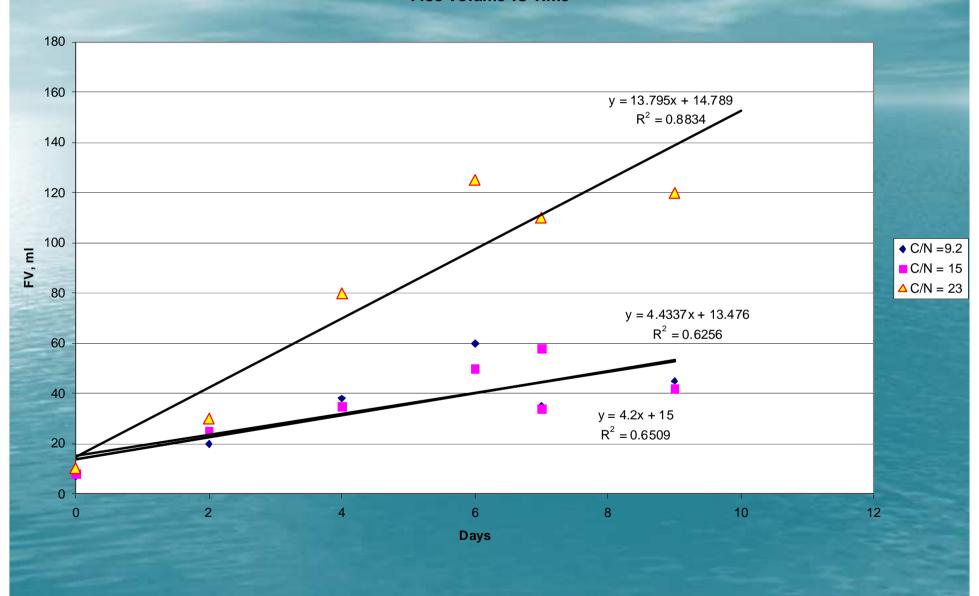
# Uptake of protein from flocs, using 15N tagging, Dor, Israel, 2005

C/N	TSS, mg/l	15N in fish (*)	Daily N Uptake mg/kg fish	Daily protein uptake mg/kg	Specific uptake (**)
9.2	441	0.3722	28.0	180	0.063
15	450	0.3725	29.2	188	0.065
23	484	0.379	<b>52.4</b>	338	0.108

<sup>\*15</sup>N(t=0) 0.3689

<sup>\*\*</sup> Daily N Uptake/TSS

#### Floc Volume vs Time



#### A lot to learn

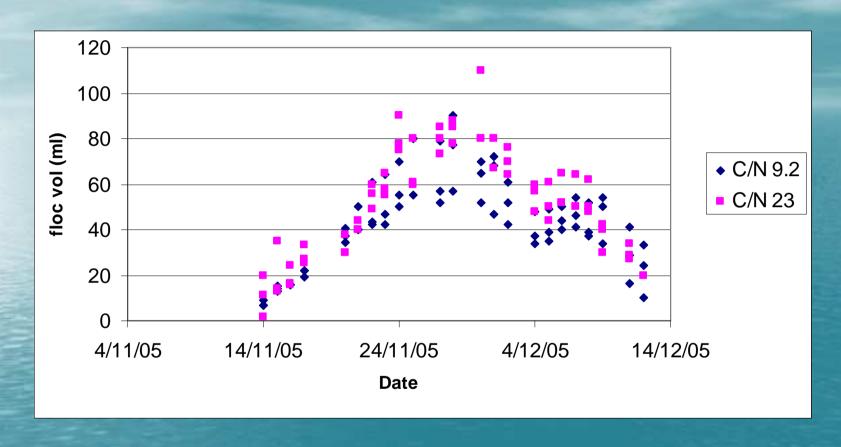
- Floc harvest rise with the increase in the size of the floc.
- How can we control it????

Does the "normal" complex microbial population in ponds effectively generate flocs? Do we need to inoculate the pond with more effective population???

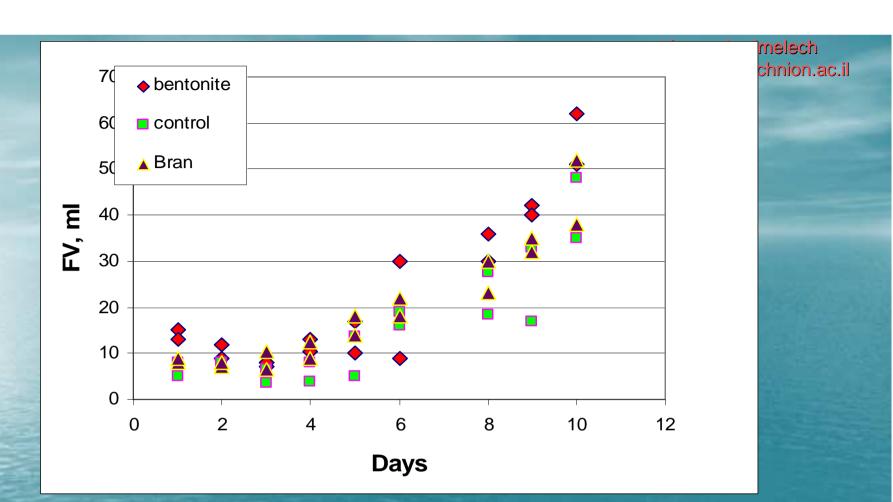


How do we get fast production of flocs? Proper C/N ratio (15-25?), Inoculation ? Addition of particles as seeds?

200 μm



Effect of C/N ratio on floc formation and stability, tank experiment. (Feeding till 28/11)



Effects of clay (bentonite, 0.04 g/l) and Bran (0.1 g/l)
On floc formation

