The truth about MaB-flocs: features, challenges and outlook

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Lab of Industrial Water and Ecotechnology (LIWET), Ghent University, Kortrijk, BE
Seminar UNESCO-IHE, Delft, NL, 9th April 2015
Outline

1. MaB-flocs: why and what?

2. Features and challenges

3. Conclusions and future outlook
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2. Features and challenges

3. Conclusions and future outlook
Microalgae bacteria pond and biomass recovery: challenges
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**Microalgae bacteria pond and biomass recovery: challenges**

**Problems:** 1. Flocculants: **cost** and **contamination** of biomass (and effluent)
Microalgae bacteria pond and biomass recovery: challenges

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   2. Centrifuge: high operation costs
Microalgal bacteria pond and biomass recovery: challenges

Problem: 1. Flocculants: cost and contamination of biomass (and effluent)
2. Centrifuge: high operation costs
3. Low strength wastewater -> only low MaB VSS possible
Microalgae bacteria pond and biomass recovery: challenges

REDESIGN: MaB-flocs
MaB-floc pond system

Microalgal bacterial floc (MaB-floc) pond
MaB-floc pond system
MaB-floc pond system

REDESIGN: MaB-flocs

Influent

Microalgal bacterial floc (MaB-floc) pond
MaB-floc pond system
**MaB-floc pond system**

**REDESIGN: MaB-flocs**

Influent  \[\rightarrow\]  Sun  \[\rightarrow\]  Supernatant  \[\rightarrow\]

Microalgal bacterial floc (MaB-floc) pond
MaB-floc pond system

REDESIGN: MaB-flocs

Microalgal bacterial floc (MaB-floc) pond

Influent → Supernatant

MaB slurry 3-8 % DM

Filter press

Discharged effluent

MaB biomass 25-50 % DM
MaB-floc pond system

REDESIGN: MaB-flocs

1. No flocculants (cost & contamination)

MaB biomass 25-50 % DM
(Milledge & Heaver, 2013)
**REDESIGN: MaB-flocs**

1. **No flocculants (cost & contamination)**

2. **Small settling tank**

**MaB-floc pond system**

- Influent
- Effluent
- Supernatant
- Microalgal bacterial floc (MaB-floc) pond
- MaB slurry 3-8 % DM
- Filter press
- MaB biomass 25-50 % DM
- Discharged effluent
- River
- Centrifuge

**MaB biomass 10-22 % DM** (Milledge & Heaver, 2013)
MaB-floc pond system

1. No flocculants (cost & contamination)
2. Small settling tank
3. Cheaper dewatering

MaB slurry 3-8 % DM

Filter press

MaB biomass 25-50 % DM

(Milledge & Heaver, 2013)
**MaB-floc pond system**

1. **No flocculants** (cost & contamination)
2. **Small settling tank**
3. **Cheaper dewatering**
4. **Low-strength wastewater & high MaB-floc VSS**

**REDESIGN: MaB-flocs**

- **Influent**
- **Supernatant**
- **Effluent**

**Microalgal bacterial floc (MaB-floc) pond**

**MaB slurry 3-8 % DM**

**Filter press**

**MaB biomass 25-50 % DM**

**Centrifuge**

**MaB slurry 3-8 % DM**

**Dischargeable effluent**

**Discharged effluent**

**River**

**Influent**

**Effluent**

**Flocculant**

**MaB biomass 10-22 % DM** (Milledge & Heaver, 2013)
MaB-flocs: from concept to pilot scale

1. MaB-flocs: why and what?

2. Features and challenges

3. Conclusions and future outlook
MaB-floc pond and biomass valorisation

1. Bioflocculation & reactor operation

Wastewater → Stirred raceway pond → MaB-flocs → Dischargeable effluent

Flue gas
MaB-floc pond and biomass valorisation

1. Bioflocculation & reactor operation

- Wastewater
- Flue gas

Stirred raceway pond

2. Wastewater treatment & flue gas

MaB-flocs

Dischargeable effluent
MaB-flocs: features and challenges

1. Bioflocculation & reactor operation

- MaB-flocs
- Wastewater
- Flue gas
- Stirred raceway pond
- Settling of MaB-flocs
- Dewatering of MaB-flocs
- Dischargeable effluent
- Biogas
- Shrimp
- Fertilizer

2. Wastewater treatment & flue gas

3. Biomass production, harvesting & valorisation
MaB-flocs: features and challenges

1. Bioflocculation & reactor operation

- Wastewater
- Flue gas
- Stirred raceway pond

2. Wastewater treatment & flue gas

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- Dischargeable effluent

3. Biomass production, harvesting & valorisation

- Biogas
- Shrimp
- Fertilizer
The secret recipe of making MaB-flocs

1. Collect microalgae & bacteria (MaB) outdoors
2. Collect wastewater
3. Mix MaB and wastewater in a sequencing batch reactor (SBR)

Selection of fast settling flocs in 1-2 weeks

The secret recipe of making MaB-flocs
Factors influencing MaB-floc settling
Factors influencing MaB-floc settling

1. A balanced OC: IC ratio of wastewater improves MaB-floc settling

IC - inorganic carbon
Filamentous flocs
Slow settling

Factors influencing MaB-floc settling

1. A balanced OC: IC ratio of wastewater improves MaB-floc settling

IC - inorganic carbon
Filamentous flocs
Slow settling

OC - organic carbon
Pin point flocs

Factors influencing MaB-floc settling

1. A balanced OC: IC ratio of wastewater improves MaB-floc settling

IC - inorganic carbon
Filamentous flocs
Slow settling

IC + OC

OC - organic carbon
Pin point flocs

Factors influencing settling of MaB-flocs

2. Calcium carbonate crystals improve MaB-floc settling
Factors influencing settling of MaB-flocs

2. Calcium carbonate crystals improve MaB-floc settling
Calcium removal from paper mill wastewater
Formation of CaCO$_3$ crystals in MaB-flocs with filamentous cyanobacteria

Van Den Hende et al., 2012b. Algaeneer Symposium Proceedings.
Factors influencing settling of MaB-flocs

3. Outdoor operation at pilot scale improves settling compared to indoor lab scale
Up-scaling of MaB-floc reactor for treatment of wastewater from pikeperch culture

4 L indoor
UGent, Kortrijk

40 L indoor MaB-floc SBR
UGent, Kortrijk
Factors influencing settling of MaB-flocs

400 L indoor MaB-floc SBR
UGent, Inagro, Roeselare

D: 0.356 m
L: 1.170 m
B: 0.960 m
Factors influencing settling of MaB-flocs

12 m³ outdoor MaB-floc SBR pond
Pilot construction by 2 Belgian SMEs:
Bebouwen & Bewaren nv: hardware
CATAEL bvba: ‘software’ automation

Settling tank
Influent tank
Effluent tank
MaB-flocs
Propellers for stirring

Factors influencing settling of MaB-flocs
Factors influencing settling of MaB-flocs

Indoor 400 L tank

Up-scaling shifted the dominant algal sp. 
Phormidium sp. indoor  
(filamentous cyanobacteria)

Outdoor pond

Ulothrix or Klebsormidum sp. outdoor  
(filamentous microalgae)

Factors influencing settling of MaB-flocs

- Up-scaling increased the crystal content
  - Red = chlorophyll -> algae
  - Blue = crystals
  - Ash in MaB-flocs was up to 30 % Ca -> CaCO₃

- Enhanced the settling of MaB-flocs
  - Correlation ash content and dSVI ($r_s = 0.935$)

- Negative for biomass valorisation?
  - Decreased energy content of biomass
  - Unbalanced Ca:P:K ratio

MaB-flocs: features and challenges

1. Bioflocculation & reactor operation

MaB-flocs

Stirred raceway pond

Wastewater

Flue gas

Settling of MaB-flocs

Dewatering of MaB-flocs

Dischargeable effluent

Biogas

Shrimp

Fertilizer

2. Wastewater treatment & flue gas

3. Biomass production, harvesting & valorisation
MaB-flocs: for which wastewaters?

Problem: wastewaters strongly differ

Solution: screen various wastewaters at lab-scale

- Sewage (Aquafin)
- Paper mill effluent (Stora Enso, VPK)
- Pike perch culture (Inagro)
- Manure treatment (Innova Manure)
- Food industry (Alpro)
- Chemical industry (BASF)

Van Den Hende et al., 2012b. Algaeneer Symposium Proceedings.
The COD, BOD$_5$, TN, NH$_4^+$, TP, PO$_4^{3-}$ concentrations were below the discharge norms.

Low PO$_4^{3-}$ and TP concentrations in effluent (< 0.8 mg TP L$^{-1}$; as low as 0.1 mg TP L$^{-1}$) -> potential as P-polishing technology.

Outdoor pond aquaculture: photosynthetic aeration

Photosynthetic aeration by microalgae was sufficient
Oversaturation
Aerobic, also at night

No mechanical aeration needed: cost saving
50% of working cost in CAS
(Conventional Activated Sludge)

10 X more land area needed!
MaB-floc ponds: 0.4 m deep
<-> CAS: 4 m deep
Outdoor pond aquaculture: pH problem

Diurnal pH fluctuations

Photosynthesis increases pH during day

Respiration decreases pH during night

Effluent discharge after night to reach pH discharge norm

-> this strategy is not sufficient in summer
Outdoor pond aquaculture: pH problem

Outdoors, CO$_2$ (5%) was needed to lower pH

- Flue gas injection = extra cost! But, low flue gas flow rates: 0.00004 vvm, so low cost

- MaB-floc SBR is not a flue gas treatment systems -> needed area is huge!
- Flue gas injection in open pond ≠ CO$_2$ credits!

*Van Den Hende et al., 2012b. Biotechnol Advances, 1405-1425.*
MaB-floc ponds for wastewater treatment: limitations

1. Pond stirring
MaB-flocs at pilot scale: 2 stirring propeller/ 30 m²  -> 10 €/kg MaB-floc TS
MaB-flocs at larger scale: 2-6 stirring propeller/ 250 m² -> 1-3 €/kg MaB-floc TS
Microalgae in HRAP of WUR (Lelystadt): 1 stirring propeller/ 250 m²
MaB-flocs: features and challenges

1. Bioflocculation & reactor operation

2. Wastewater treatment & flue gas

3. Biomass production, harvesting & valorisation

- MaB-flocs
- Wastewater
- Flue gas
- Stirred raceway pond
- Settling of MaB-flocs
- Dewatering of MaB-flocs
- Dischargeable effluent
- Biogas
- Shrimp
- Fertilizer
Biomass productivity: what is realistic?

Problem:
Lab results are hard to translate to outdoor results

Aquaculture wastewater
Lab PBR
160 ton
MaB-floc VSS ha\(^{-1}\) pond \(y\)\(^{-1}\)

Outdoor ponds
? ton
MaB-floc VSS ha\(^{-1}\) pond \(y\)\(^{-1}\)
Biomass productivity: what is realistic?

Problem:
Lab results are hard to translate to outdoor results

Solution:
Up-scaling to MaB-floc raceway pond

Aquaculture wastewater
Lab PBR

160 ton

MaB-floc VSS ha\(^{-1}\)\_pond y\(^{-1}\)

Outdoor ponds

? ton

MaB-floc VSS ha\(^{-1}\)\_pond y\(^{-1}\)
### Scale-up decreased the biomass productivity

10 times less TSS, 13 times less VSS

Average outdoors: $33 \, \text{ton TSS ha}^{-1} \text{y}^{-1}$ or $12 \, \text{ton VSS ha}^{-1} \text{y}^{-1}$

1.5-4 times lower compared to ww-fed HRAP in New Zealand (Park et al., 2011)

But, no optimisation yet!

### Table: Reactor TSS and VSS Productivity

<table>
<thead>
<tr>
<th>Reactor</th>
<th>TSS productivity (mg TSS L_{reactor} day^{-1})</th>
<th>VSS productivity (mg VSS L_{reactor} day^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 L indoor</td>
<td>$236 \pm 73 , (10)^* $</td>
<td>$109 \pm 30 , (13)$</td>
</tr>
<tr>
<td>40 L indoor</td>
<td>$65 \pm 8 , (2.9)$</td>
<td>$45 \pm 6 , (5.5)$</td>
</tr>
<tr>
<td>400 L indoor</td>
<td>$16 \pm 23 , (0.6)$</td>
<td>$12 \pm 17 , (1.3)$</td>
</tr>
<tr>
<td>12 m³ outdoor</td>
<td>$23 \pm 54 , (1.0)$</td>
<td>$8 \pm 18 , (1.0)$</td>
</tr>
</tbody>
</table>

TSS: total suspended solids; VSS: volatile suspended solids; *Scale-up conversion factor

MaB-floc harvesting: 1. settling, 2. filtering

1. Settling of MaB-flocs (1 hour)

2.1. Filtering by gravity

2.2. Filtering by hydropress

MaB-floc SBR pilot

MaB-floc cake
MaB-floc harvesting: **99%** biomass recovery!

1. Concentrating step: settling

MaB-floccs in pond

Supernatant:
7.9 ± 5.7 % MaB-floc TSS loss, pumped back into pond - > **No loss!**

Settled MaB-floc slurry 70 g TSS L⁻¹
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2. **Dewatering step: filtering 150-250 µm**
   2.1. **Gravity filtering**
   - Gravity filtrate: 1.2 ± 0.9 % MaB-floc TSS loss
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   2.1. **Gravity filtering**
   - Gravity filtrate: 1.2 ± 0.9 % MaB-floc TSS loss

   2.2. **Hydropress filtering**
   - MaB-floc cake: 43 ± 8 % dry matter
   - Press filtrate: 0.05 ± 0.03 % MaB-floc TSS loss
Biomass production and harvesting: conclusions

Features

1. Moderate biomass productivity

2. Very efficient and cost-effective MaB-floc harvesting
   Pilot: water-powered filter press (4 bar water): 0.16 € kg\(^{-1}\) DM MaB-flocs
   Com.: electricity-powered filter press: 0.04-0.07 kWh\(_{el}\) kg\(^{-1}\) DM (Udom et al., 2013)
   -> Dewatering: < 0.01 € kg\(^{-1}\) MaB-flocs TSS!

Limitations

1. Proof-of-principle needed for other wastewaters
Biogas: conclusions

AD to biogas seems not economically interesting for these MaB-flocs

Biogas price is low: < 0.01 € m\(^{-3}\) wastewater treated
Low compared to wastewater treatment cost of 0.30-0.60 € m\(^{-3}\) (Verstraete et al., 2009)

Practical constraints
Scaling (CaCO\(_3\)) of reactors due to high ash content of MaB-flocs

Needed: biomass valorisation pathways with €
If MaB-floc market price of 2.5 € kg\(^{-1}\) DM -> 0.32 € m\(^{-3}\) wastewater

Van Den Hende et al., 2014d. XI Simposio Latinoamericano de Digestion Anaerobia, 11/24-27, La Havana.
Shrimp feed: MaB-floc inclusion?

Problem:

What to do with these low-energy MaB-flocs?

Research question:

Can MaB-flocs be included in diets of white Pacific shrimp? *Litopenaeus vannamei* (Boone 1931)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash</td>
<td>62</td>
</tr>
<tr>
<td>Calcium</td>
<td>17</td>
</tr>
<tr>
<td>Protein</td>
<td>21</td>
</tr>
<tr>
<td>Lipid</td>
<td>4</td>
</tr>
</tbody>
</table>

0 - 2 - 4 - 6 - 8 % inclusion
Mainly wheat was replaced

1. Shrimp quantity?
2. Shrimp quality?
Shrimp feed: no effect on production

0-2-4-6-8 % MaB-floc inclusion did NOT lead to significant differences of:

- **Shrimp quantity**
  - Survival: 93 - 97 %
  - Weight gain: 0.32 - 0.34 g day\(^{-1}\)
  - Feed conversion ratio: 1.17 - 1.27

- **Shrimp quality of shrimp tails**
  - Proximate composition
  - Fatty acid profile

**Shrimp feed: no effect on production**

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**Shrimp feed: significant effect on pigmentation**

MaB-flocs effect pigmentation of cooked shrimp tails
Enhanced redness and yellowness

MaB-flocs contain 0.25 %m carotenoids

Increased market value of shrimp?

Van Den Hende et al., 2014c. Aquaculture Research, DOI:10.1111/are.12564.
Shrimp feed: EU market regulations – is it legal?

Regulation EC No 767/2009: **NO!**
Restricts the use of faeces and urine including of fish (aquaculture) in feed that enters the EU market
Restricts the use of waste from treatment of industrial wastewater in animal feed

Directive 91/271/EEC: **BUT…**
Industrial wastewater is ‘wastewater which is discharged’
e.g. aquaculture wastewater in closed RAS = process water?

Opportunities: **YES?**
Don’t bring it on the market -> use it where it is produced -> integrated system
Process water free of urine and manure particles

But, more research is needed
Health of shrimp and consumers
Environmental sustainability: LCA
Environmental sustainability: LCA study

Poster of Sophie Sfez et al. (Ugent) @ Algae around the World
Potential for system improvement & biomass valorisation

Algae Around the World Symposium, Cambridge, United Kingdom

Forecasting the environmental sustainability of a microalgae raceway pond treating aquaculture wastewater: from pilot plant to system integration at industrial scale

Sophie Sfez¹, Sofie Van Den Hende², Sue Ellen Taelman¹, Steven De Meester¹, Jo Dewulf¹
¹Department of Sustainable Organic Chemistry and Technology (EnVOC), Faculty of Bioscience Engineering, Ghent University
²Laboratory for Industrial Water and Eco-Technology (LIWET), Faculty of Bioscience Engineering, Ghent University

Introduction

Recirculating aquaculture systems (RAS) produce nutrient-rich effluents which need to be treated:
- Fish sludge: treatment by anaerobic digestion is an attractive option (Mirzoyan et al., 2010)
- Backwash water supernatant: innovative microalgal bacterial floc sequencing batch reactor (MaB-floc SBR) technology (Van Den Hende et al., 2014a) is a promising way to avoid costly mechanical aeration of conventional activated sludge systems
  → Harvested MaB-flocs can be valorized as shrimp feed or as biogas (Van Den Hende et al. 2014b, 2014c)

Switching from linear fish aquaculture and separated aquaculture sludge and wastewater treatment to an integrated MaB-floc-based aquaculture waste treatment system could be a key strategy to mitigate the environmental footprint of the aquaculture sector

How can the environmental sustainability of existing MaB-floc technology be improved?
How should MaB-flocs be valorized when the technology is implemented in an aquaculture waste treatment system?

Material and methods

1. To study the improvement potential of the MaB-floc SBR raceway pond technology:
- Data collection from a pilot MaB-floc raceway pond (28 m²) treating backwash wastewater, sunlight, and MaB-floc harvested from the system in two microalgal cultures: Dunaliella tertiolecta and Botryococcus braunii
- Data analysis and modeling for system optimization and energy recovery
Environmental sustainability: biogas vs shrimp

Resource footprint of shrimp feed is lower than for biogas

$U_p_L$: 1 ha linear up-scaled 40 ponds of 250 m$^2$

CEENE 2014: Cumulative Exergy Extraction from the Natural Environment (Dewulf et al., 2007)

Shrimp feed: conclusions

- Low inclusion of MaB-flocs in shrimp diets enhances shrimp pigmentation

- LCA: shrimp feed preferred above biogas

- Implementation potential is limited
  - In NWE Europe: integrated systems, manure-free wastewater
  - Tropical regions with a large shrimp industry -> New green water technology
Fertilizer: MaB-flocs?

Problem:
What to do with these low-energy MaB-flocs?

Research question:
MaB-flocs as slow-release fertilizer? 
Mix with substrate for tomato hydroculture

Differences in:
1. Plant growth?
2. Mineralisation rate?
3. Leaves and fruits?

Drs Joeri Coppens
16.00 - 16.30
The application of microalgae as a slow-release fertilizer: tomato cultivation as a model
Outline

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MaB-floc SBR ponds: conclusions

Features
Very promising results on bioflocculation: better outdoors than lab-scale
Proof-of-principle of year round photosynthetic aeration in NW Europe
Very efficient and cost-effective harvesting

Limitations
Area and heat -> (sub)TROPICS
Stirring -> Optimisation & demonstration scale
MaB-floc SBR ponds: conclusions

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Research needs
ECO: economic study and greenhouse gas emissions
Outdoor screening other wastewaters
SBR or continuous stirred reactor & settling tank (Germany, Spain, New Zealand)
Biomass valorisation -> Key to unlock the potential of MaB-floc SBR technology
In 2014, 2 wastewaters:
- UASB effluent
- CAS effluent

1. Outdoor pilot at Alpro food company (UGent LIWET)

2. Economics (UGent Economy & LIWET)

3. Biomass valorisation (UGent LIWET)

Settling of MaB-flocs
Dewatering of MaB-flocs
Dischargeable effluent
MaB-floc SBR
Flue gas
Biogas
High value compounds?

EnAlgae research until 2015/09
The truth about MaB-flocs: Eye-opening outdoor results
The truth about MaB-flocs: Eye-opening open freezer door results

Liquid fraction of dewatered MaB-flocs after (accidently) thawing
EnAlgae research until 2015/09

Valorisation of MaB-flocs grown on wastewater of food company Alpro
Aphanocapsum sp. or Aphanotheca sp.

Interested in a collaboration?
2 paid master student positions available July-September 2015
sofie.vandenhende@ugent.be
Thank you

The truth about MaB-flocs: a work of many people, and many more to come

Alpro: Coolsaet Carlos, Vanhoucke Thomas
Aquaculture Farming Technology: Claessens Leon
Bebouwen en Bewaren nv: Bourez Lode, Bourez Val
CATAEL bvba: Capoen Henk, Taelman Nikolas, Tanghe Niels
Crevetec: De Muylder Eric
Ghent University - LIWET: Beelen Veerle, Laurent Cedric, Rousseau Diederik, Van Den Hende Sofie, Vervaeren Han + Master students: Bégue Marine, Bore Gaëlle, Desmet Sem, Carrré Erwan, Coaud Elodie, Julien Lucy, Laurent Cedric, Lefoulon Alexandra, Rodrigues Andre, Sanczuk Anouk
Ghent University - LabMET: Boon Nico, Coppens Joeri, Grunert Oliver
Ghent University - LET: De Gelder Leen, Haesaert Geert
Ghent University - ENVOC: De Meester Steven, Dewulf Jo, Sfez Sophie, Taelman Sue-Ellen
Ghent University - PAE: Jeroen Van Wichelen
Inagro: Bourgeois Geert, Buyse Laurens, Teerlinck Stefan
Aquafin, BASF, Havatex, Howest, Innova Manure, VPK, Stora Enso, ...
EnAlgae is a strategic initiative of the INTERREG IVB North West Europe (NWE) Programme

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