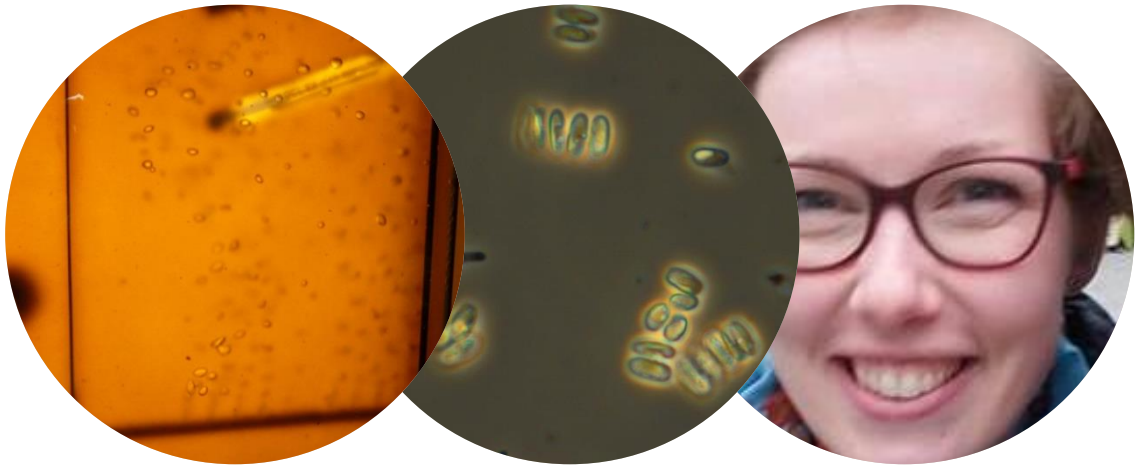
Continuous versus batch production of microalgal lipids

Ilse Remmers, René Wijffels, Packo Lamers

Bioprocess Engineering – AlgaePARC
Wageningen University, the Netherlands



WAGENINGEN UR
For quality of life

fuel4me

The problem: the lipid yield gap

	TAG yield on light (g mol_{ph}^{-1})
Theoretical maximum	**1.33***
Current best practice (large scale, outdoor, wild type)	**0.10 – 0.15****
Empirical maximum (best wild types, optimal laboratory conditions)	**0.26 – 0.35*****

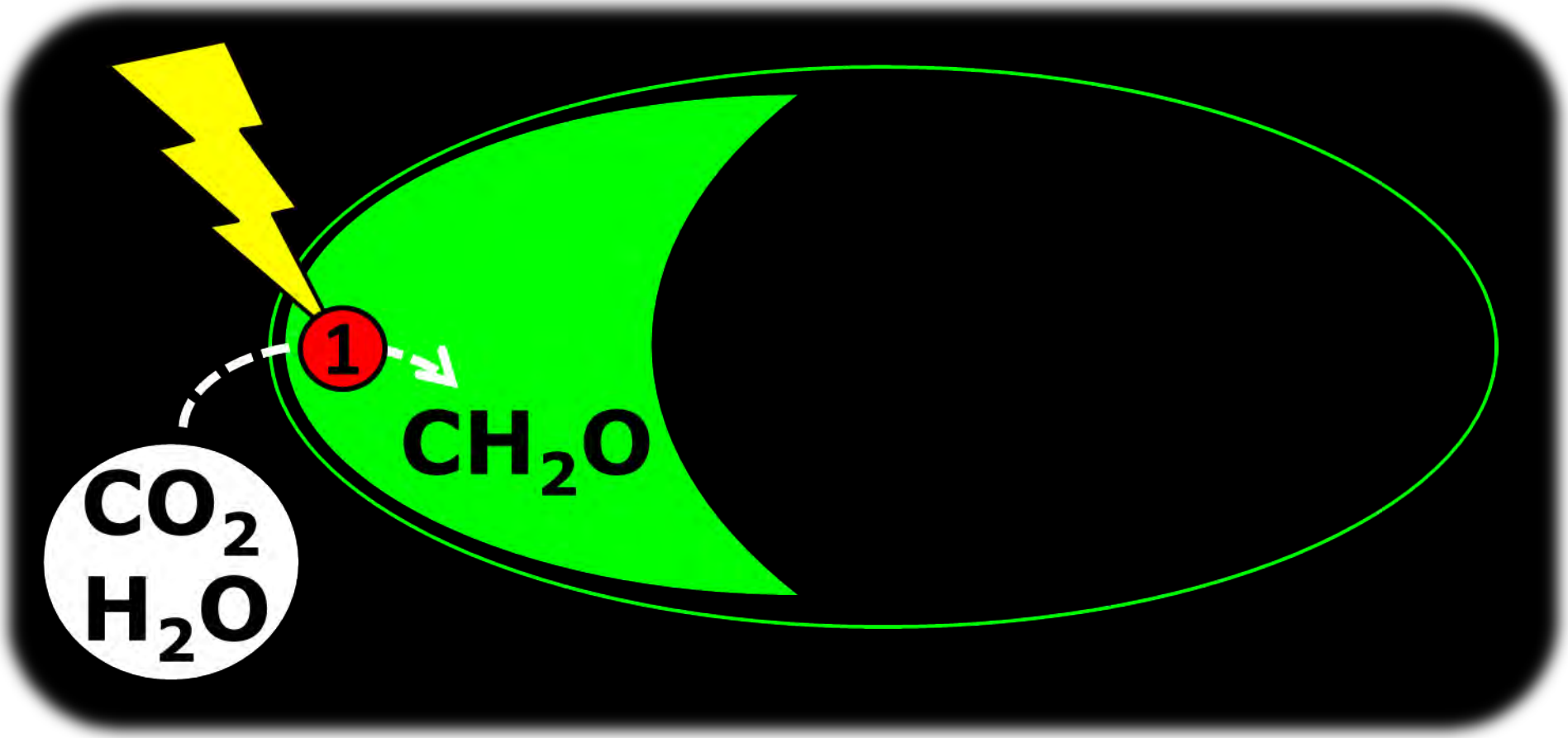
* - Breuer *et al.*, 2015 *Bioresource Technology* 186: 294-302

** - Quinn *et al.*, 2012 *Bioresource Technology* 117: 164-171
- Benvenuti *et al.*, 2015, *Biotechnology for Biofuels* 9:100

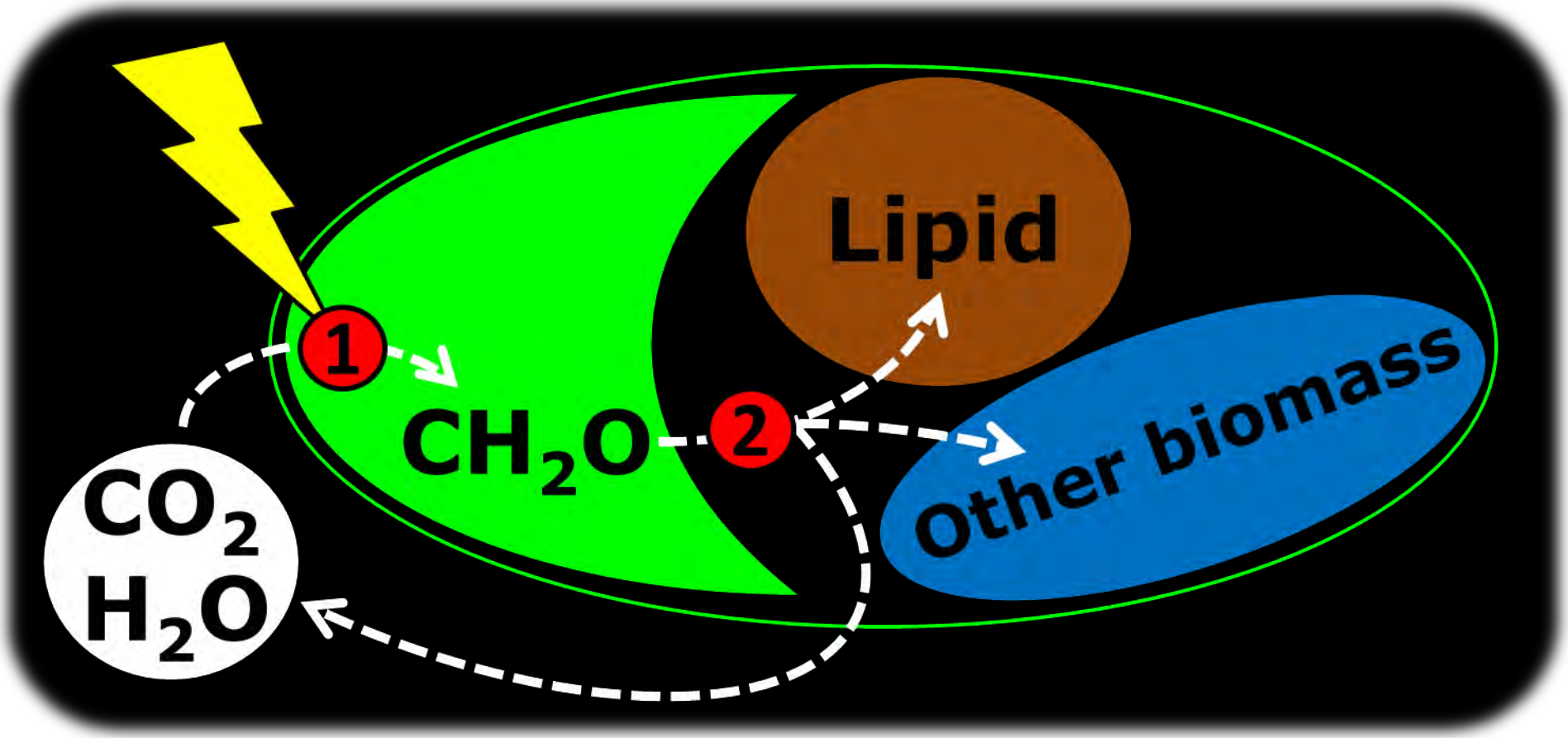
***- Breuer *et al.*, 2012 *Bioresource Technology* 124: 217-226
- Mulders *et al.*, 2014 *Algal Research* 6: 8-16
- Breuer *et al.*, 2013 *Bioresource Technology* 143: 1-9

WAGENINGEN UR
For quality of life

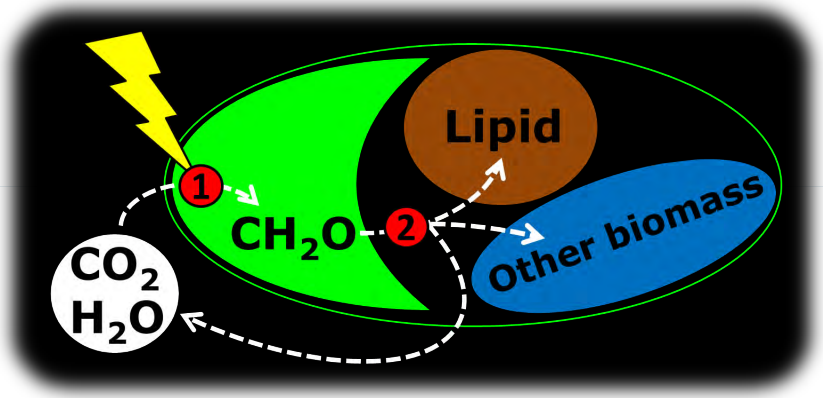
Problem: two key ‘processes’



Problem: two key 'processes'



Underlying problems



Low photosynthetic efficiency	Losses	1.33 g/mol
• *Inherent losses of photochemistry*	*20%*	*1.07*
• *Suboptimal light & temp.*	*60%*	*0.43*
• *N-starvation*	*50%*	*0.21*

Suboptimal carbon distribution		
• *Starch, structural carbohydrates, other lipids*	*30%*	*0.15*

WAGENINGEN UR
For quality of life

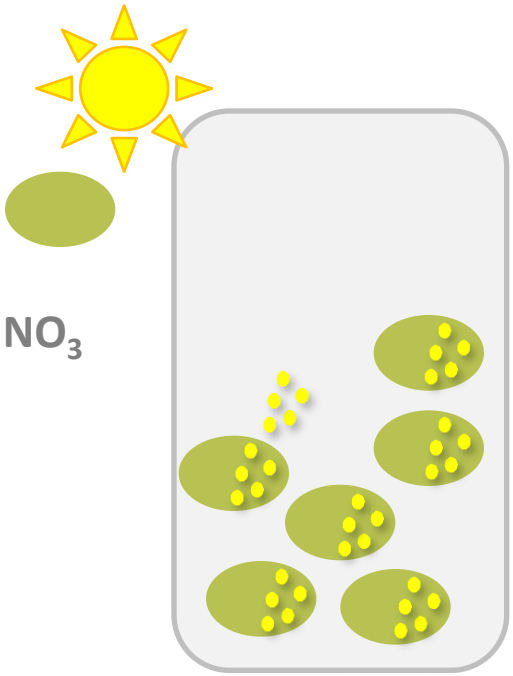
fuel4me

Breuer *et al.*, 2015 *Bioresource Technology* 186: 294-302

Batch versus continuous cultivation

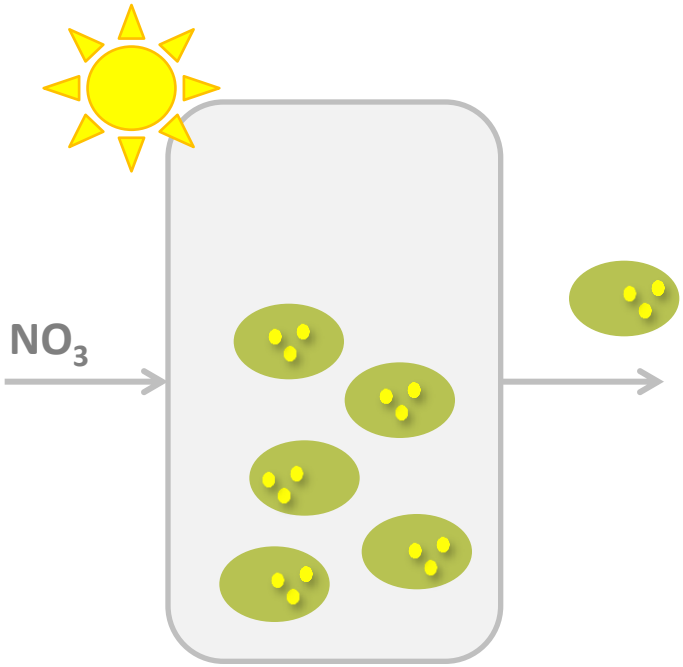
Batch

Starvation



Continuous

Limitation

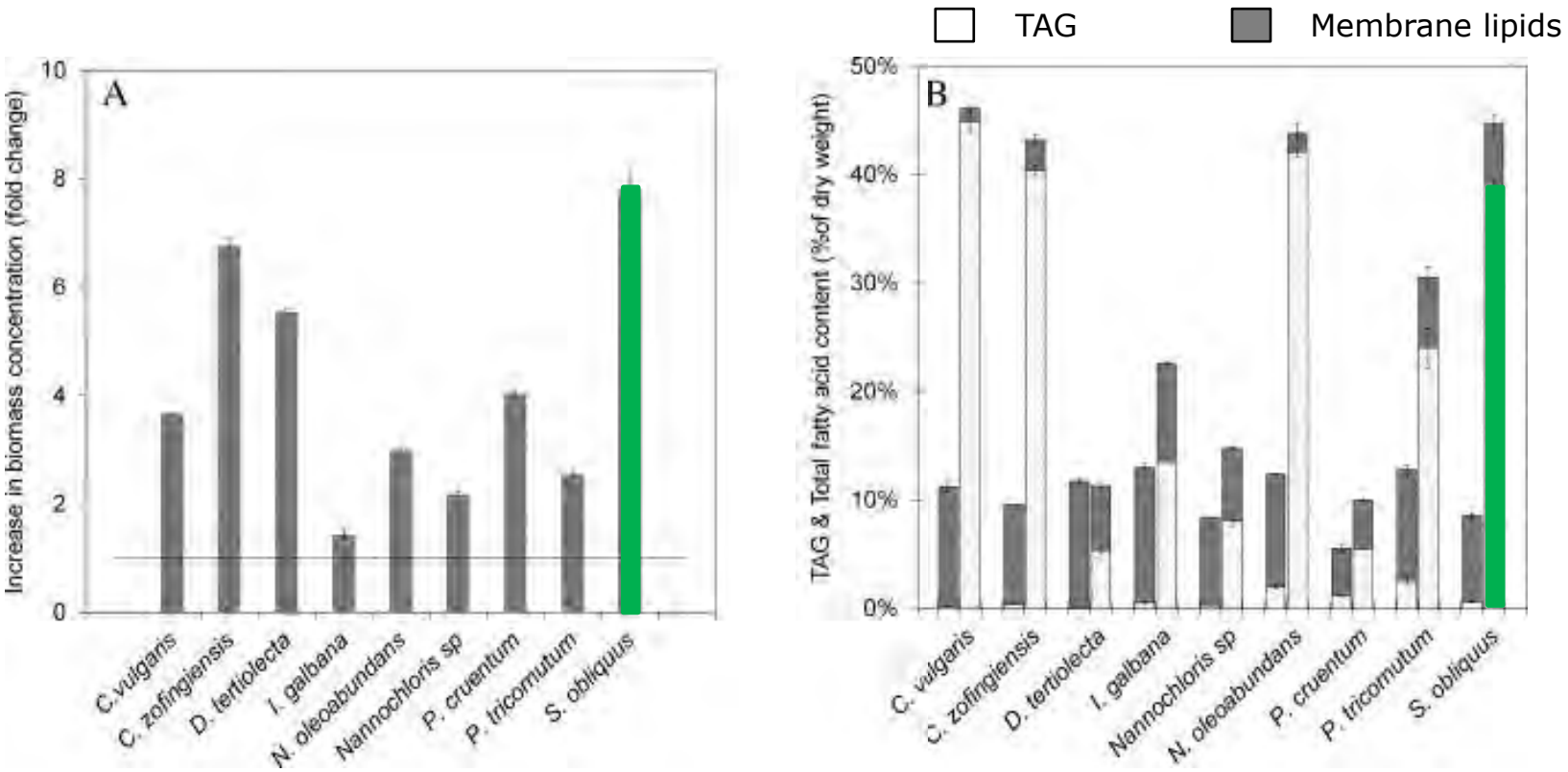


WAGENINGEN UR
For quality of life

fuel4me

Selecting the right species

Maintaining high PE in a continuous process



Bonus: starchless mutant with 50% increased TAG yield on light

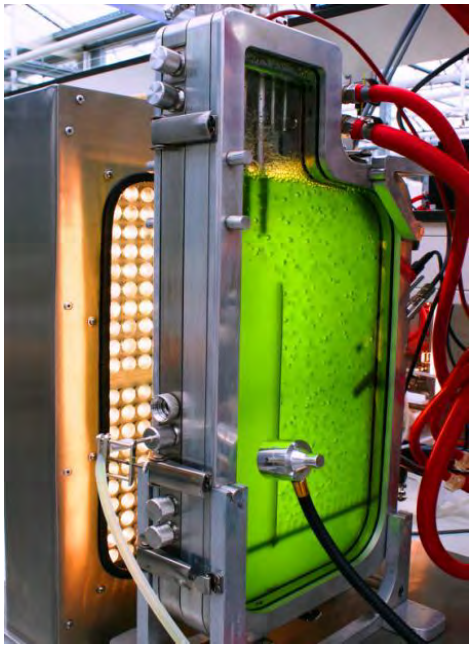
WAGENINGEN UR
For quality of life

Unilever

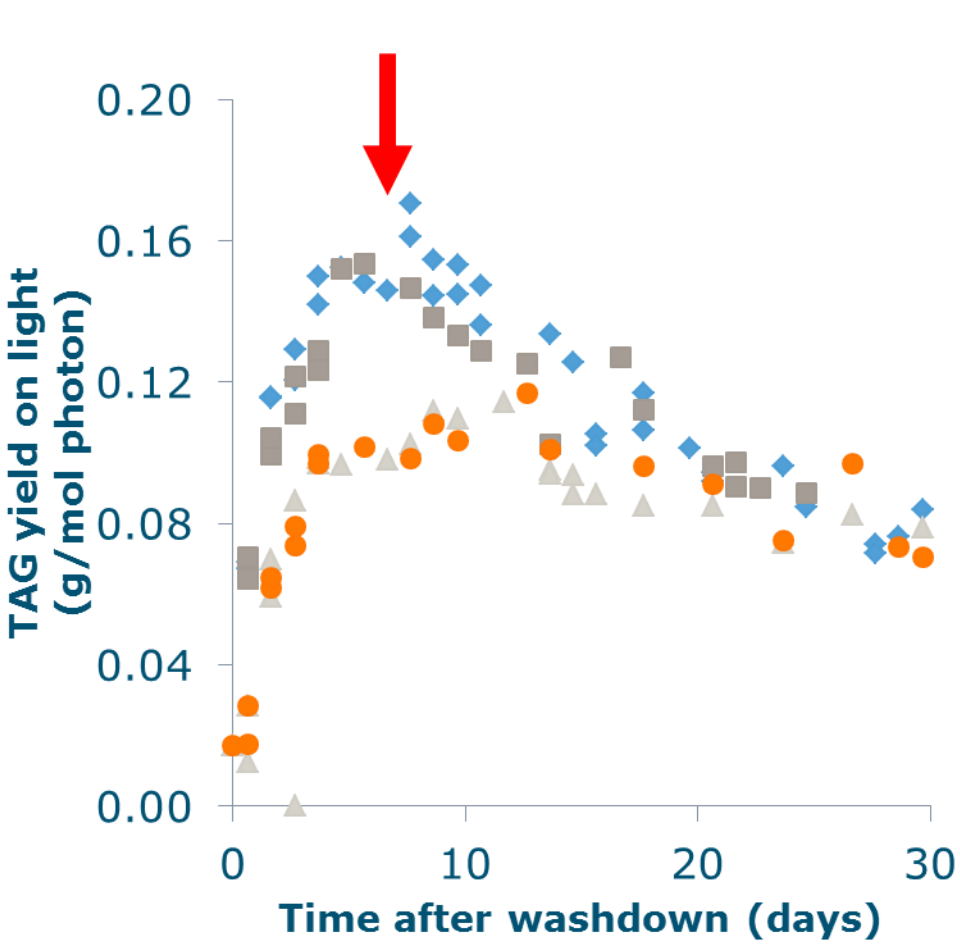
Breuer *et al.*, 2012 *Bioresource Technology* 124: 217-226
De Jaeger *et al.*, 2014 *Biotechnology for Biofuels* 7:69
Breuer *et al.*, 2014 *Biotechnology for Biofuels* 7:70

Batch vs continuous production - approach

- **Two strains**
  - *S. obliquus* wildtype (starch producer)
  - *S. obliquus* starchless mutant slm1
- **Batch cultivations**
  - DN cycles (16h:8h light/dark)
  - Nitrogen washdown at 1.5 g/L biomass
- **Continuous cultivations**
  - DN cycles (16h:8h light/dark)
  - Turbidostat/nutristat operation
  - Different N feed rates (100, 75, 50, 35, 15% of demand)



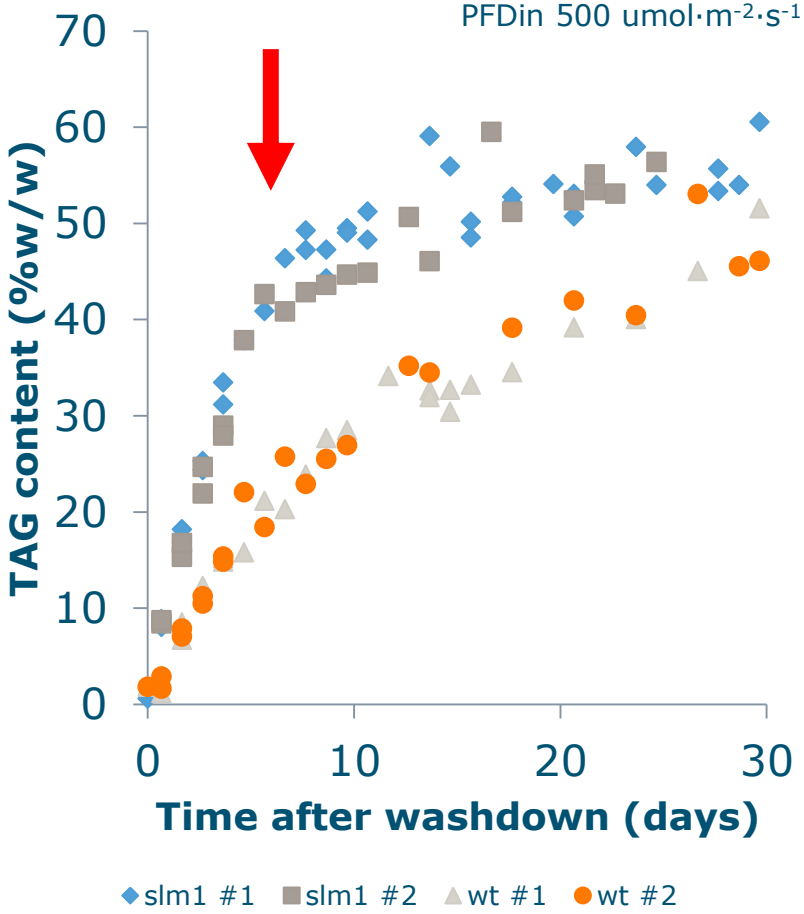
Batch lipid production



S.obliquus

DN cycle (16:8)

PFDin 500 umol·m^{-2}·s^{-1}



WAGENINGEN UR
For quality of life

fuel4me

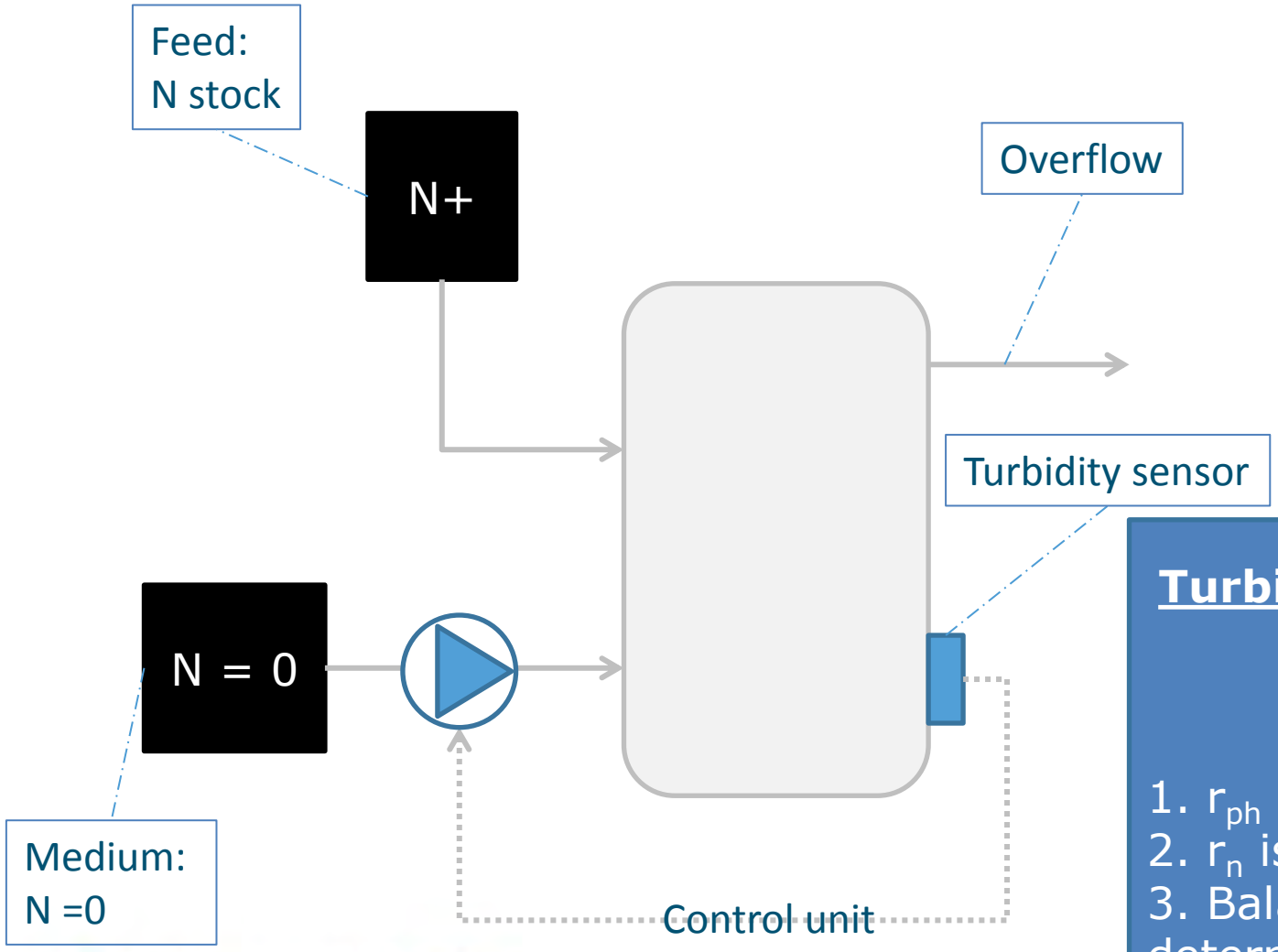
Batch lipid production

S.obliquus
DN cycle (16:8)
PFDin 500 umol·m^{-2}·s^{-1}

	S. obliquus **Wild type**	***S. obliquus*** **slm1**
Max TAG yield on light* (g/mol photon)	0.11	0.16
Max TAG productivity* (g/L.day)	0.16	0.23
TAG content ** (%w/w)	33	40

* Time averaged (Calculated between t and moment of starvation) and corrected for light needed for the production of 1.5 g/L initial biomass
** At point of max TAG yield on light

Experimental setup - Continuous cultivations

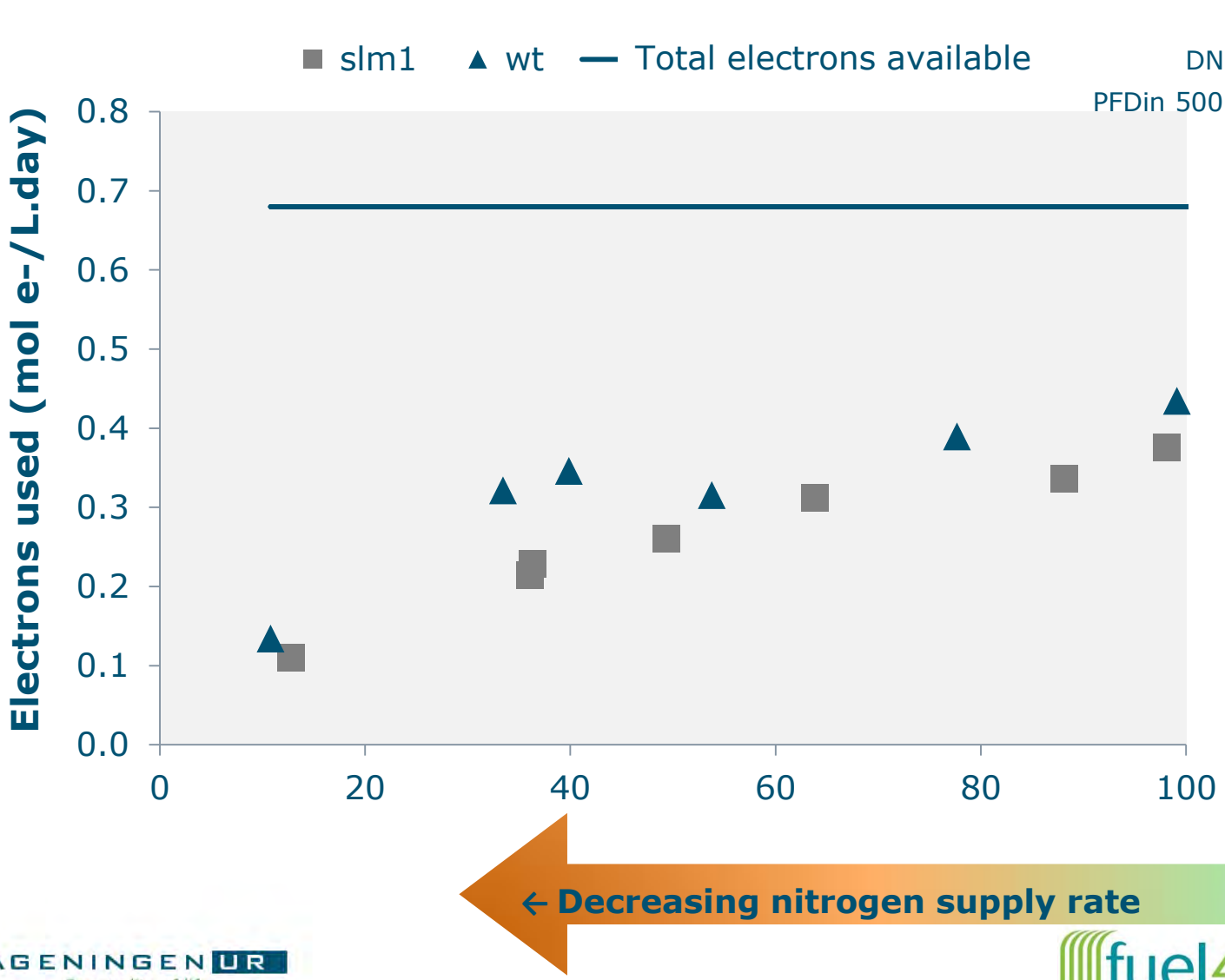


Turbidostat/nutristat control

1. r_{ph} is fixed
2. r_n is fixed
3. Balance of both rates determines stress level

WAGENINGEN UR
For quality of life

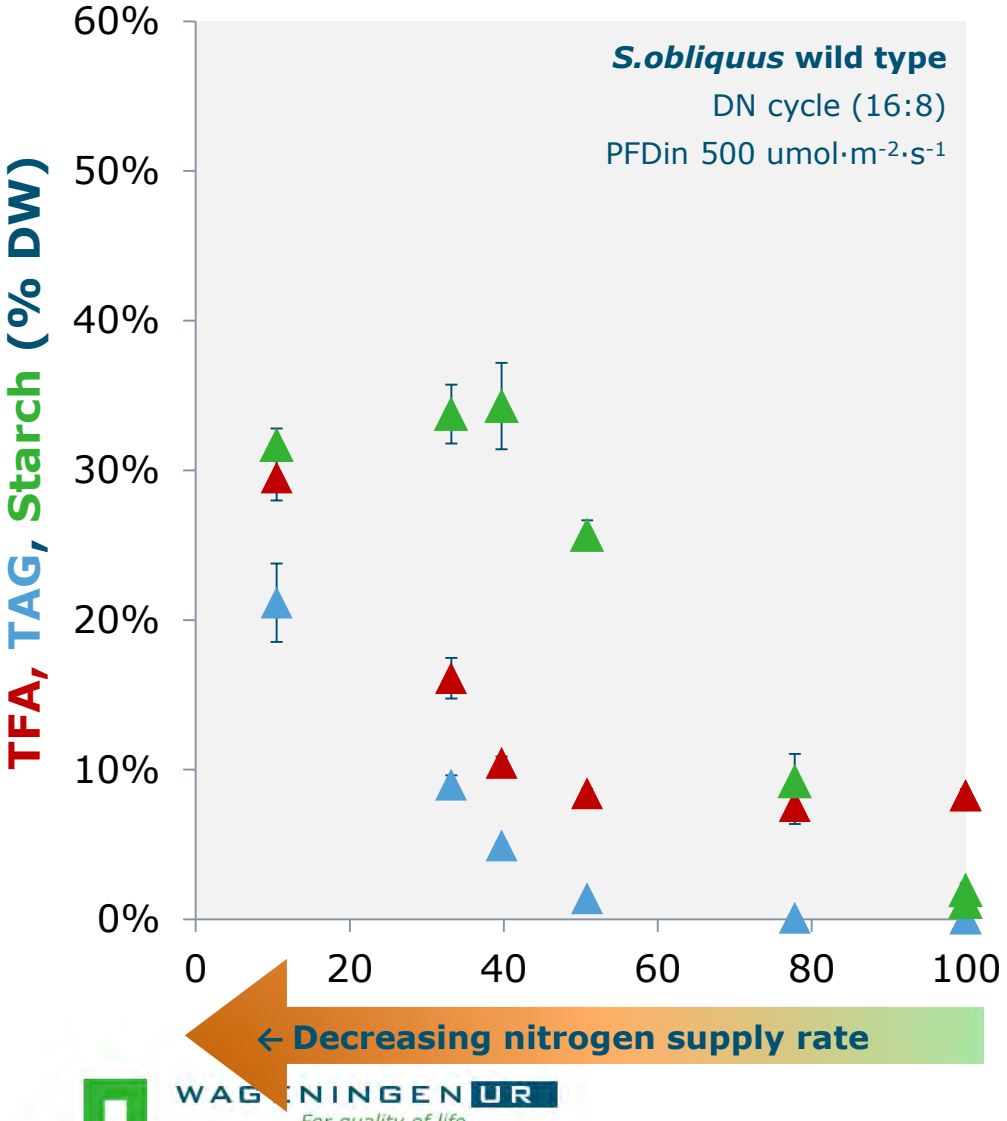
Photosynthetic efficiency (continuous)

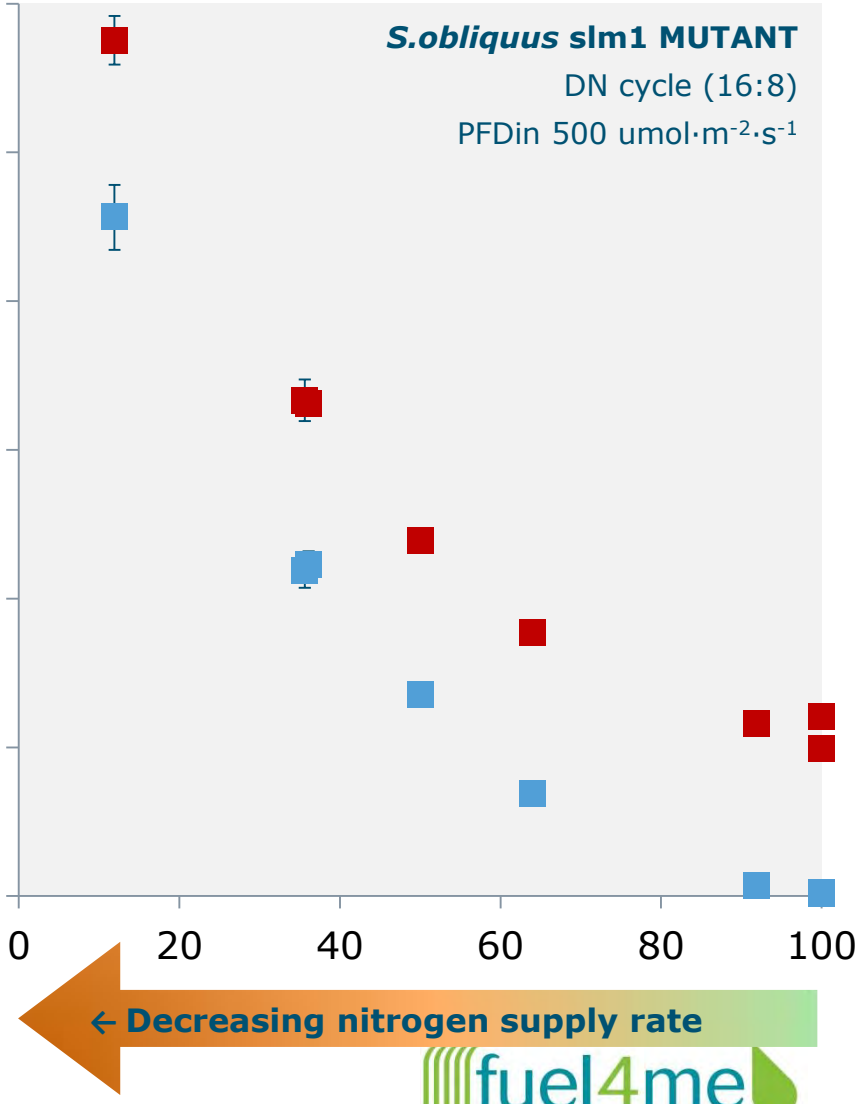


WAGENINGEN UR
For quality of life

fuel4me

Accumulation of lipids and starch (continuous)



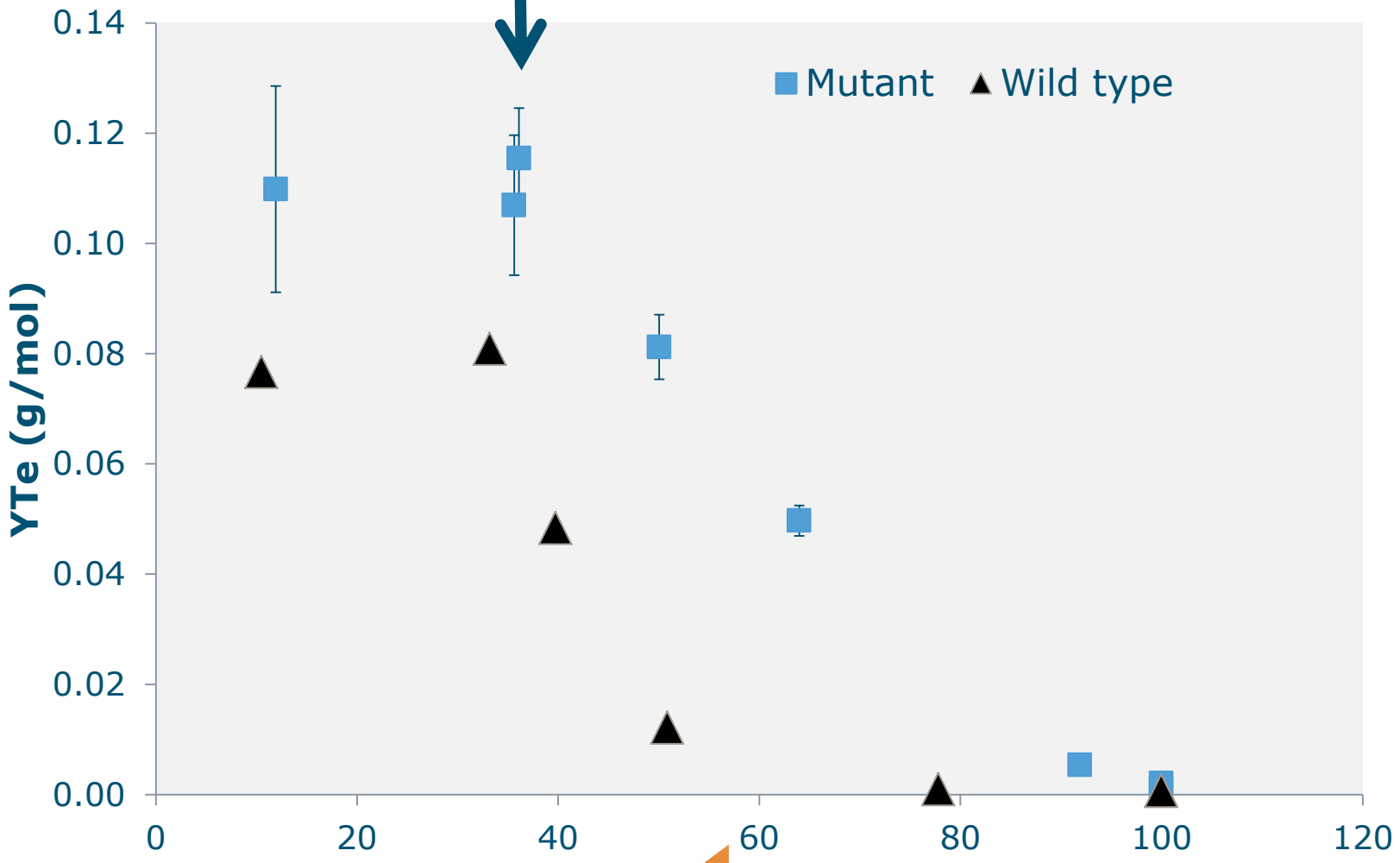


WAGENINGEN UR
For quality of life

fuel4me

TAG yield on light (continuous)

S.obliquus
DN cycle (16:8)
PFDin 500 umol·m^{-2}·s^{-1}



← Decreasing nitrogen supply rate

WAGENINGEN UR
For quality of life

Summarizing: batch vs. continuous

	S. obliquus Wild type		*S.obliquus* slm1	
	Batch	Continuous	Batch	Continuous
Max TAG yield on light (g/mol photon)	0.11*	0.08	0.16*	0.12
Max TAG productivity (g/L.day)	0.16*	0.12	0.23*	0.17
TAG content (%w/w)	33**	18	40**	40

* Time averaged (Calculated between t and moment of starvation) and corrected for light needed for the production of 1.5 g/L initial biomass
** At point of max TAG yield on light

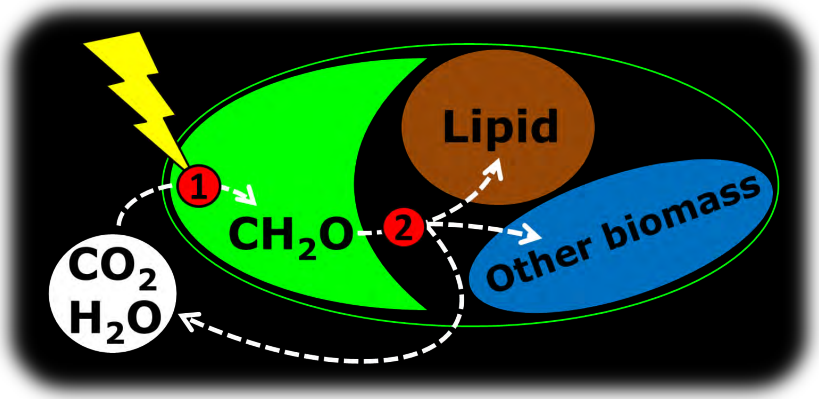
WAGENINGEN UR
For quality of life

fuel4me

Current challenges of algal lipid production

Low photosynthetic efficiency

- *Inherent losses of photochemistry*
- *Suboptimal light & temp.*
- *N-starvation*



Suboptimal carbon distribution

- *Starch, structural carbohydrates, other lipids*

Strain improvement

- *Industrially relevant strains*

WAGENINGEN UR
For quality of life

fuel4me

Acknowledgements

Ilse.Remmers@wur.nl, Adrian Hidalgo, Boudewijn Brandt, René Wijffels

Courses:

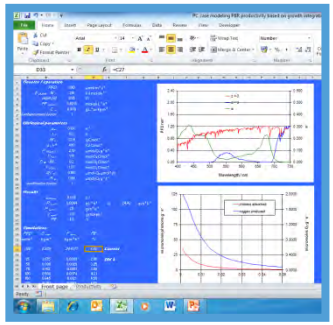
Microalgae Process Design

8-15 July 2016, AlgaePARC, Wageningen

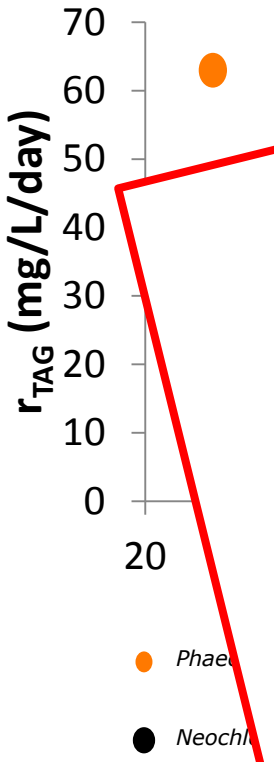
Microalgae Biorefinery

18-20 July 2016, AlgaePARC, Wageningen

www.vlaggraduateschool.nl/courses/



Potential of One step lipid production



Important to improve capacity to convert photons into biomass and lipids

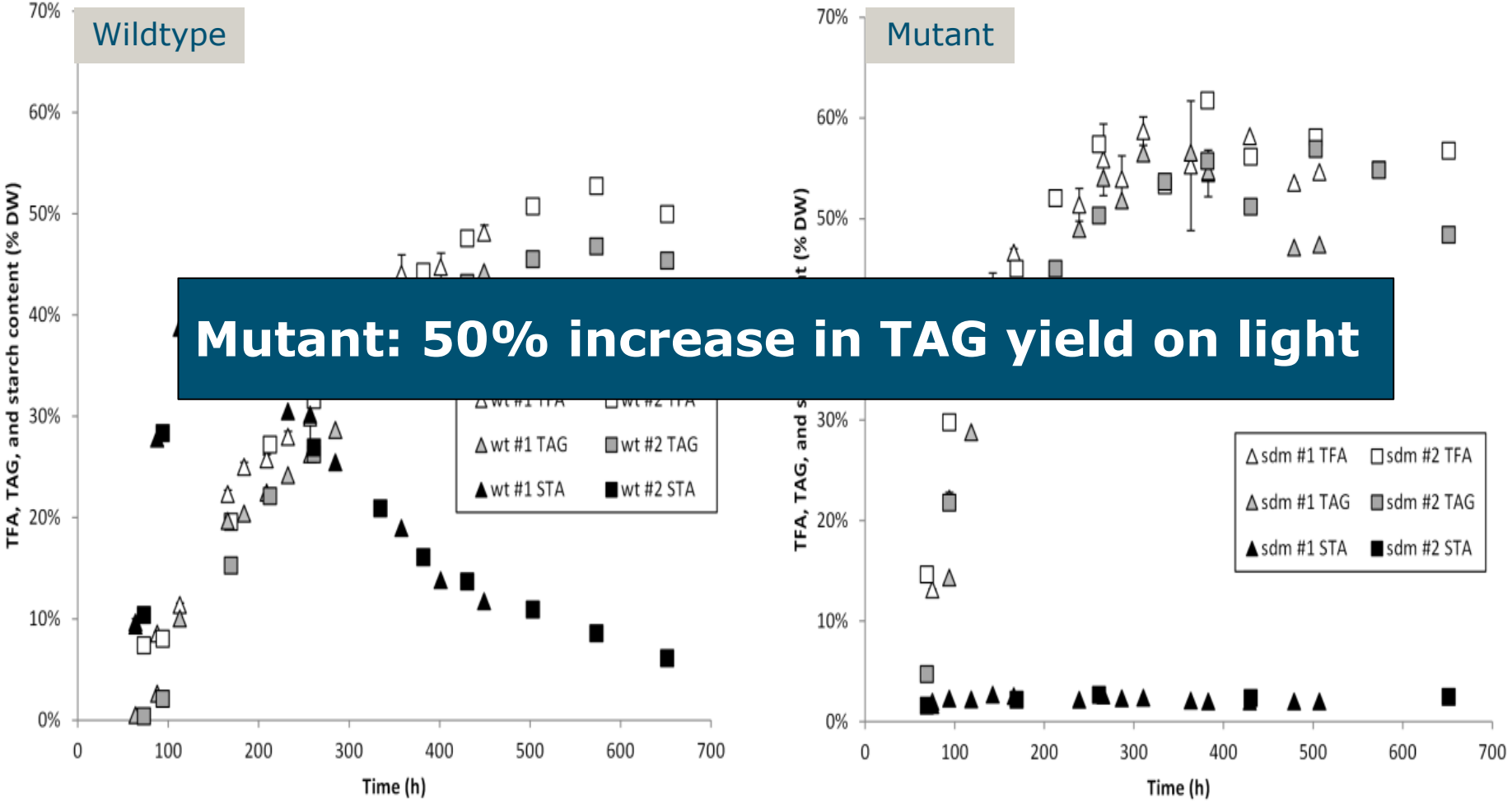
- **Strain improvement → high PE**
- **New strain selection**

ction:

	atch	
		250 umol DN 16:8h
	0.063	**0.049**
Y TAG.E (g/mol ph)	0. 087	**0.021**

Selecting the right species

Improved carbon partitioning in a starchless mutant



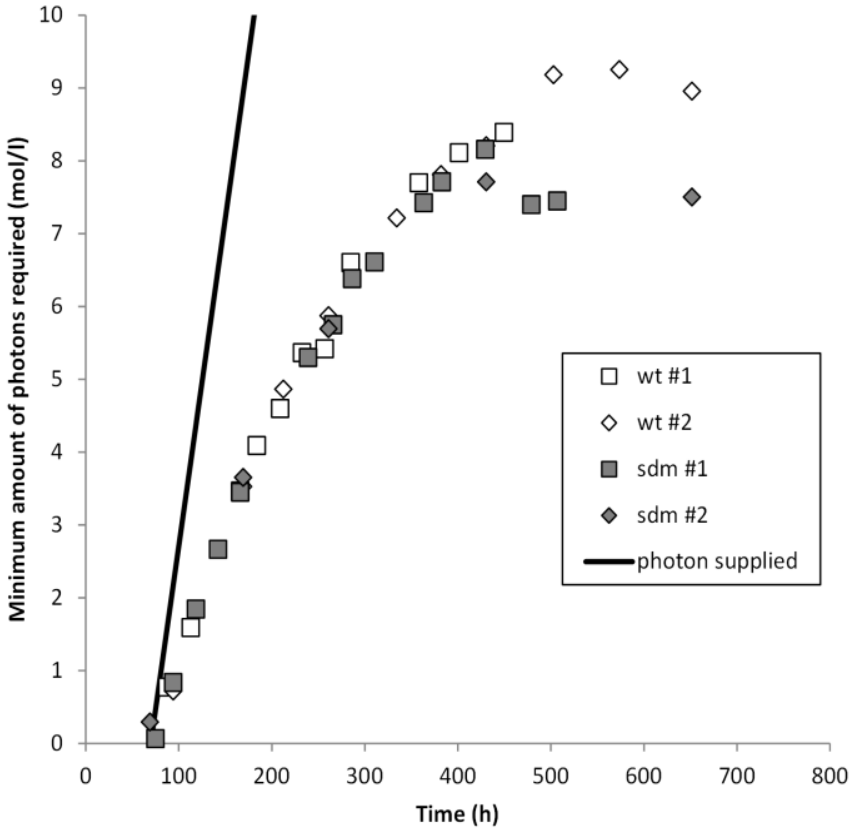
Mutant: 50% increase in TAG yield on light

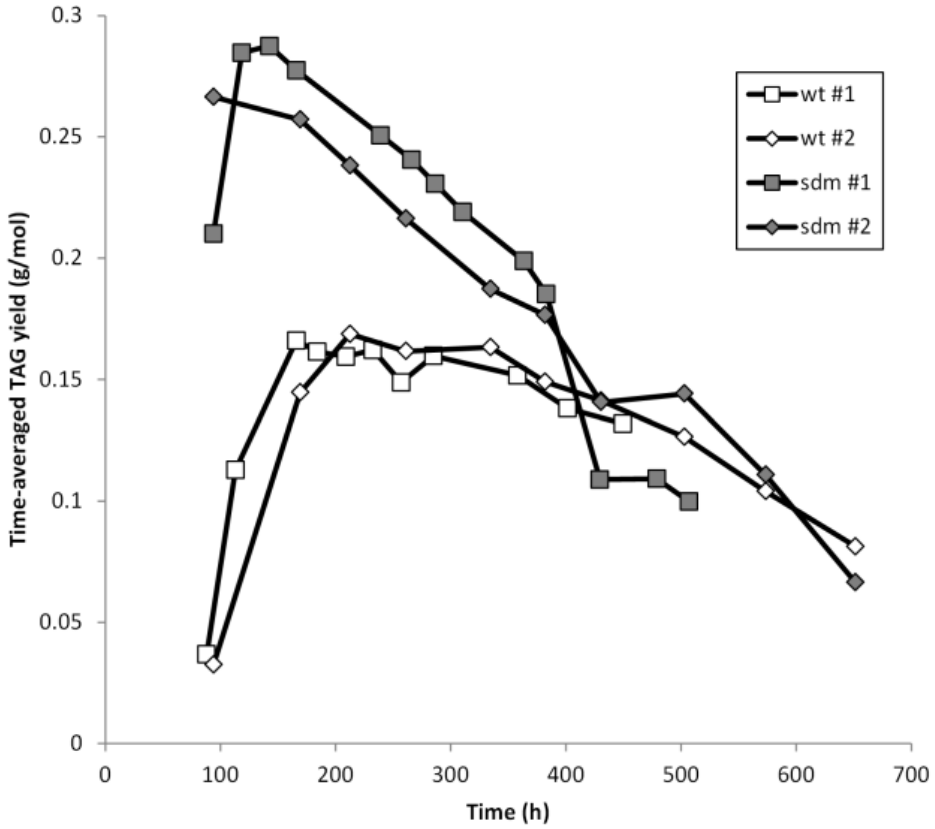
WAGENINGEN UR
For quality of life

Unilever

De Jaeger *et al.*, 2014 *Biotechnology for Biofuels* 7:69
Breuer *et al.*, 2014 *Biotechnology for Biofuels* 7:70

Mutagenesis – Starchless mutant





Mutant: 50% increase in lipid yield

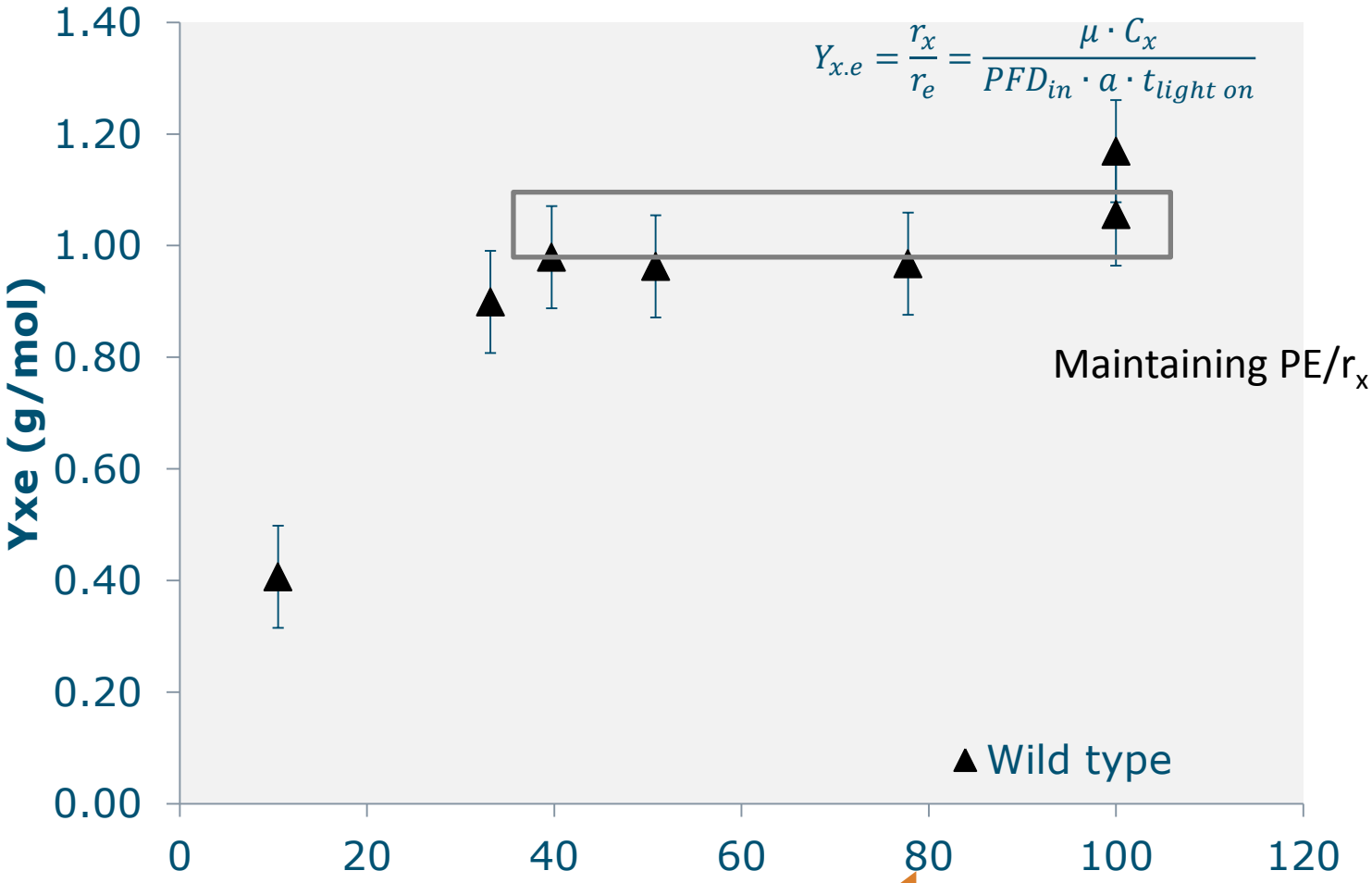
WAGENINGEN UR
For quality of life

Unilever

De Jaeger *et al.*, 2014 *Biotechnology for Biofuels* 7:69
Breuer *et al.*, 2014 *Biotechnology for Biofuels* 7:70

Continuous lipid production

S.obliquus
DN cycle (16:8)
PFDin 500 umol·m^{-2}·s^{-1}



← **Decreasing nitrogen supply rate**

WAGENINGEN UR
For quality of life

fuel4me