An Approaching Global Phosphorus Crisis and Microalgal Biotechnology:

A Growing Problem & Strategies for Effective Use

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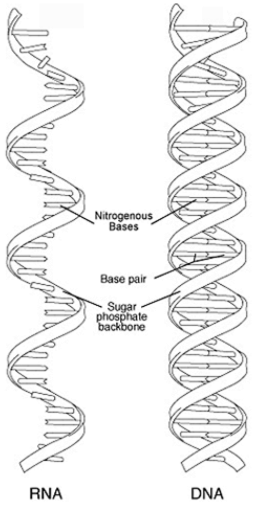
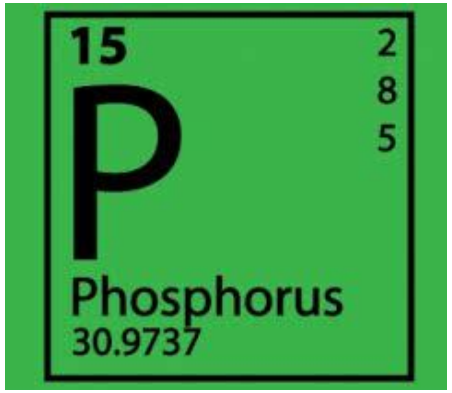
Biology of P - Where and why?





Essential macronutrient:

Maintains membrane structure

Synthesis and expression of genetic material

Energy metabolism

Regulatory processes

RNA > phospholipids > DNA > P-esters > Inorganic P

Total P content

Microalgae	0.2 – 2% % DW (but upto 3.2% DW – luxury uptake)
Corn & wheat	0.2 – 1.5 % DW
Coffee beans	~ 0.4 % DW
Saccharomyces	0.2 – 1.6 % DW

So why worry about phosphorus?

Finite mineral resource + Non-even distribution + Environmental impacts

(Morocco & Western Sahara (74%), China (6%), Algeria (3%), Syria (3%))

Open-cast/strip mining & Processing

Large areal requirement

Soil erosion & desertification

Altered groundwater aquifers

Large water demand

Rock phosphorus mine, Togo, Africa. Photo: Alexandra Pugachevskaya

Land-use change

Ecosystem destruction & biodiversity loss

Eutrophication

Hydrofluoric gas emission

United Nations Environment Programme, 2001; Kuo & Muñoz-Carpena, 2009; Watson, et al., 2014; Kalmykova, et al., 2015.

So why worry about phosphorus?

World reserves ~ **67,000,000 Mt**

World production in 2014 ~ **225 Mt yr^{-1}**

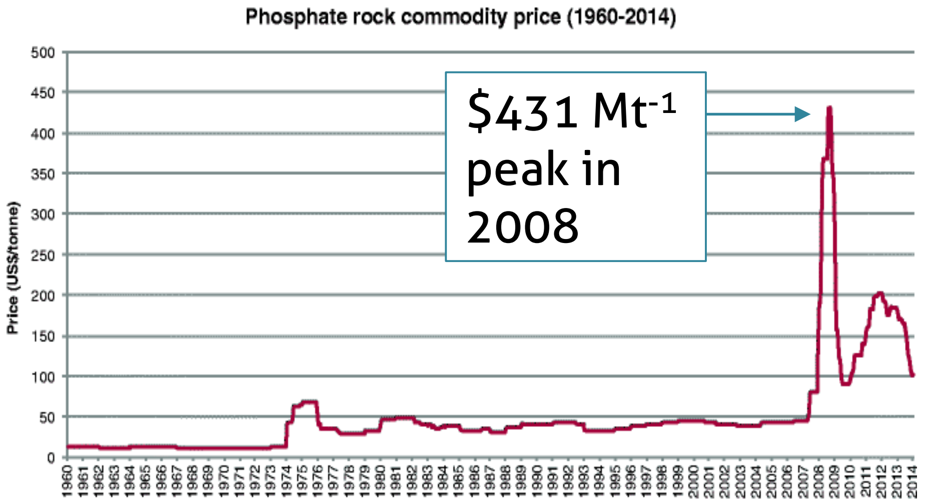
Peak production in **30 – 100 yrs**.

> 80% of P used in fertilizers…

Monoammonium phosphate, DAP NH_4PO_4

Diammonium phosphate, MAP $(NH_4)_2PO_4$

Triple super phosphate, TSP $Ca(H_2PO_4)_2$



$431 Mt^{-1} peak in 2008

Phosphate rock commodity price 1960–2014. Data source: World Bank Commodity Price Data (historical). http://go.worldbank.org/4ROCCIEQ50.

P fertiliser (% P)	**Energy / kg** (Mj kg P or N^{-1})	**Cost / kg** (\$ kg P or N^{-1})	**GWP potential** (kg CO_2-eq kg P or N^{-1})
MAP (27)	56.2	4.24	0.81
DAP (21)	73.8	3.11	1.54
TSP (25)	58.9	2.93	3.30
NH_4NO_3	51.0	1.71	9.37

Calculated from Johnson, et al., 2013 and Handler, et al., 2012. GWP potential calculated using Ecoinvent 2013.

Van Kauwenbergh, 2010; Cordell & White, 2014; Jasinski, 2014.

So why worry about phosphorus?

Irresponsible agricultural and
waste management practices =
Large eutrophication potential

Nutrient run-off → Algal blooms →

Fishery degradation

Human health risks

Economic loss

Decoupled P cycle

Deoxygenation of the Baltic Sea during the last century

Jacob Carstensen[a,1], Jesper H. Andersen[a], Bo G. Gustafsson[b], and Daniel J. Conley[c]

Hypoxia Is Increasing in the Coastal Zone of the Baltic Sea

Daniel J. Conley,[*,†] Jacob Carstensen,[‡] Juris Aigars,[§] Philip Axe,[‖] Erik Bonsdorff,[⊥] Tatjana Eremina,[#] Britt-Marie Haahti,[⊥] Christoph Humborg,[$,@] Per Jonsson,[@] Jonne Kotta,[%] Christer Lännegren,[▽]

GULF OF MEXICO HYPOXIA, A.K.A. "THE DEAD ZONE"

Record-setting algal bloom in Lake Erie caused by agricultural and meteorological trends consistent with expected future conditions

A broken biogeochemical cycle

Excess phosphorus is polluting our environment while, ironically, mineable resources of this essential nutrient are limited. **James Elser** and **Elena Bennett** argue that recycling programmes are urgently needed.

…gal blooms in the Baltic
…005. Jeff Schmaltz, NASA

Satellite image of an alg…
USA in 2011. Jeff Schmaltz, National Ocea…
and Atmospheric Administration/NASA

Prud'homme, 2010; Elser & Bennett, 2011; Cordell & White, 2015.

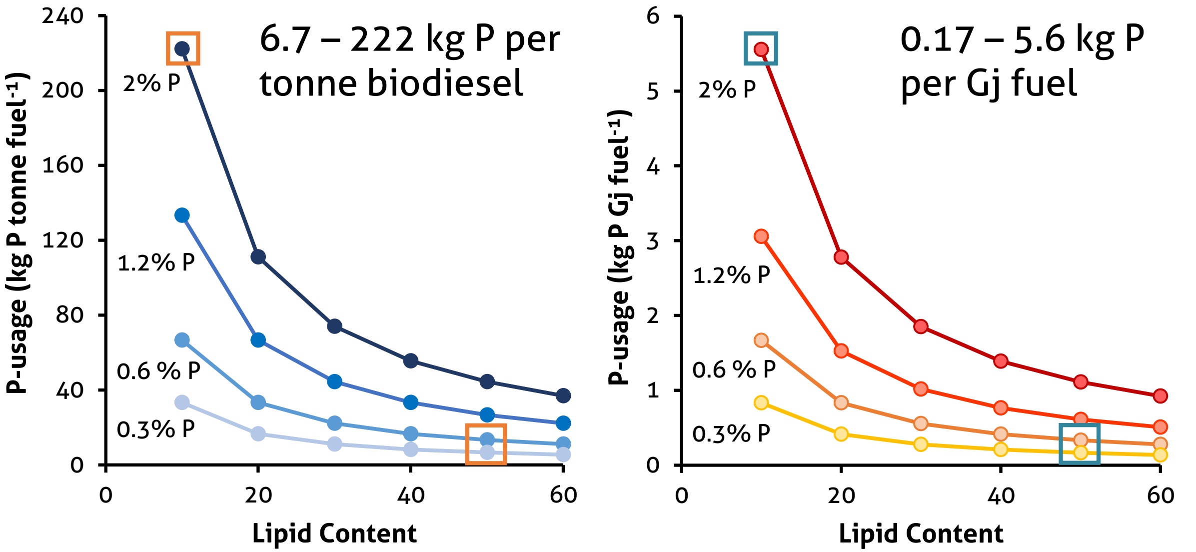
Phosphorus in alga-culture

Large cultivations are few & often not detailed = Reuse of non-representative numbers

Contained production means low release of P to environment + more efficient resource use.

Biomass	kg P per tonne DW^{-1}	kg P per tonne fuel^{-1}	kg P per Gj fuel^{-1}
Microalgae	3.1 – 20		
Soybean	8.1 – 14.2[a]	79 – 192 [b]	2.0 – 4.8
Canola	3.2 – 15.7[a]	16 – 42.1 [b]	0.4 – 1.1
Sunflower	9 – 47.3[a]	35.3 – 186 [b]	0.9 – 4.7
Corn	6.5 – 8.8[a]	15.7 – 20 [c]	0.6 – 0.7

[a] amount applied to production area required to produce 1 metric tonne, some lost to run-off; [b] biodiesel, fuel density = 40 Mj kg^{-1}; [c] ethanol, fuel density = 26.8 Mj kg^{-1}



Effect of biomass P content and lipid content on P requirements for production of 1 tonne fuel or 1 Gj energy. Assumes 90% of lipid converted to biodiesel, with energy content = 40 Mj kg^{-1}.

Literature: 0.6 – 1.5 kg P per Gj fuel^{-1} (Pate, et al., 2011, Redford ratio of 106:16:1; 1.2% P)

Pimentel & Patzek, 2008; Cavalett & Ortega, 2009; Chen & Chen, 2011; Fazio, et al., 2011; Handler, et al., 2012; Malca, et al., 2014; Beal, et al., 2015.

Phosphorus in alga-culture

Pate, et al., 2011:
Algal biofuel production would consume **20 - 51%** of annual US P-fertilizer consumption to produce 38 billion litres (28% of USA EISA 2007 target)

Canter, et al., 2015:
34 – 53% to produce 19 billion litres (23% of USA EISA 2007 target).

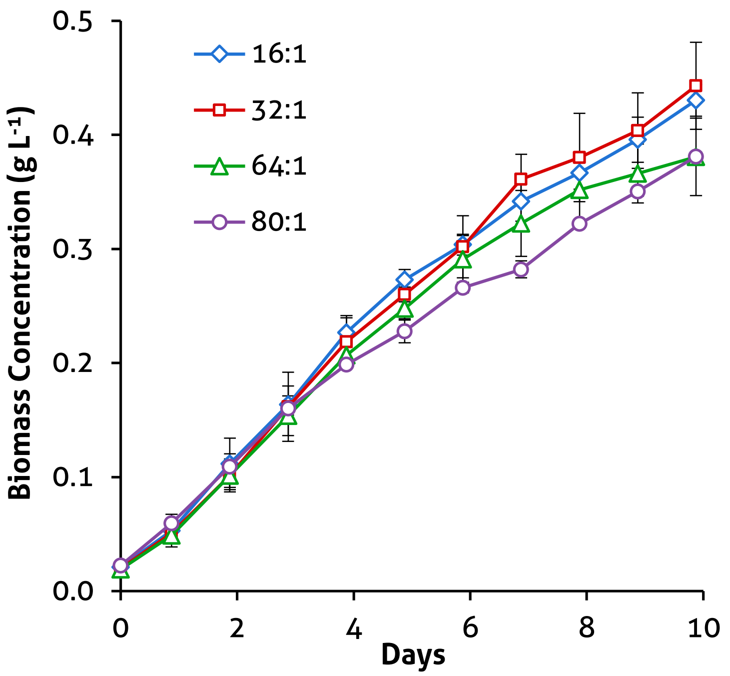
→ Contribute significantly to the negative energy balance for biofuel production.

→ Concept of bioenergy production not feasible with current model of fertilizer usage.

STRATEGIES NEEDED TO REDUCE RELIANCE ON COSTLY FERTILIZERS

Optimising P-usage – N:P ratio

Nannochloropsis sp. cultured at different media N:P ratios in batch culture



Biomass production *by Nannochloropsis* sp. grown at different N:P ratios in batch culture.

Growth parameters of *Nannochloropsis* sp. batch cultures grown at different N:P supply ratios (n = 3, mean = 1SD)

N:P ratios	16:1	32:1	64:1	**80:1**
Exp. Growth rate (d^{-1})	0.62 ± 0.01^{a}	0.62 ± 0.02^{a}	0.60 ± 0.01^{a}	0.56 ± 0.01^{b}
Max. DW Prod. (mg L^{-1} d^{-1})	56.6 ± 2.2^{a}	52.3 ± 0.4^{ab}	50.5 ± 1.5^{b}	45.4 ± 0.5^{c}
N content (% DW)	2.6 ± 0.2^{a}	2.6 ± 0.1^{a}	2.9 ± 0.1^{b}	2.9 ± 0.1^{b}
P content (% DW)	0.24 ± 0.02^{a}	0.16 ± 0.01^{b}	0.10 ± 0.01^{c}	0.08 ± 0.01^{c}

Significantly different treatments are represented by different letters (One-way ANOVA with Tukey post hoc, $p < 0.05$).

Lipid content = 49 – 52% DW

Increase media N:P ratio to 64:1 without significant negative effects.

> 64:1 N:P, reduced growth rate, biomass production and lipid productivity.

P content down to 0.1% DW.

See Mayers, et al., 2014.

Optimising P-usage – N:P ratio

Nannochloropsis **biomass nutrient requirements**

Lipid content (% DW)	kg N per tonne DW	kg P per tonne DW	kg N per tonne fuel	kg P per tonne fuel	kg P per Gj fuel
N-starved, P-replete					
20	60	2.5	333	13.9	0.35
50	30	2.5	67	5.6	0.14
N-starved, low-P					
20	60	1.0	333	5.6	0.14
50	30	1.0	67	2.2	0.06

Literature: 0.6 – 1.5 kg P per Gj fuel^{-1}

(Pate, et al., 2011; Redfield ratio of 106:16:1; 50% C, 8.8% N, 1.2% P)

Significantly lower requirement than predicted using Redfield stoichiometry !!

Could reduced further with a P-starved system.

Models need to consider flexible C:N:P ratios

However, N still > 80% energy demand of macronutrient requirements in media

Reduced footprint of P-usage

N and P media comparison.

Based on use of ammonium nitrate and triple-super phosphate

Biomass state	N & P Content (% DW)	Lipid content (% DW)	Per tonne DW^{-1}		Per tonne fuel^{-1}		% of biomass energy content for N + P (just P) [c]
			Cost ($)	Energy (Gj)	Cost ($)	Energy (Gj)	
Redfield[a]	8.8 / 1.2	20% [b]	153	5.3	852	29.5	22.2% (3.4)
Replete	6 / 0.5	20%	94	3.4	537	18.9	14.2 % (1.4)
Minimum	3 / 0.1	50%	42	1.6	93	3.55	6.7% (0.3)

[a] assumes a 50% C content; [b] not determined, but predicted to be likely content; [c] the higher heating valu of algal biomass = 24 Gj tonne DW^{-1}

N is more significant contributor to media cost and energy.

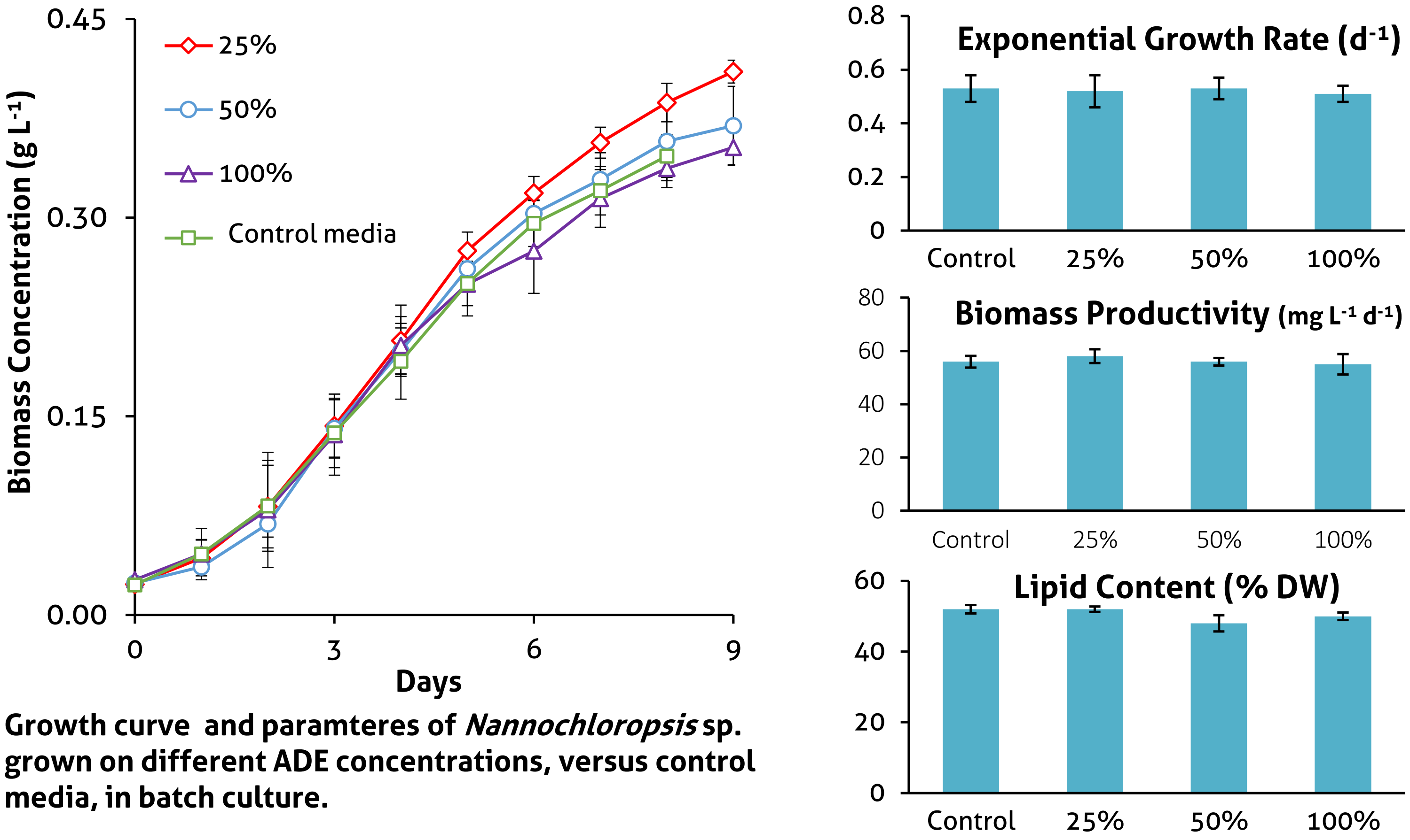
Cost and energy saving related to media N & P use compared to Redfield media

Per tonne DW	Cost savings (%)	Energy saving (%)
Ratio increase	72.8	69.9

Reducing fertilizer usage – Waste nutrients

Nannochloropsis sp. grown on anaerobic digestate effluent (ADE) to replace N.

ADE = **1.6** g NH_4 L^{-1} & **0.036** g PO_4 L^{-1} (NP = 99:1)



Growth curve and paramteres of *Nannochloropsis* sp. grown on different ADE concentrations, versus control media, in batch culture.

ADE can replace **100%** of media N !!

Also tested at 32:1 and 64:1 ratios successfully.

For biomass of 3% N and 0.1% P, ADE use reduces P fertilizer input by **67%**.

Need to consider cost of sterilizing ADE. May limit applications of biomass.

Reduced footprint of nutrient usage

N and P media comparison.

Based on use of ammonium nitrate and triple-super phosphate

Biomass state	N & P Content (% DW)	Lipid content (% DW)	Per tonne DW^{-1}		Per tonne fuel^{-1}		% of biomass energy content for N + P (just P) [c]
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Redfield[a]	8.8 / 1.2	20% [b]	153	5.3	852	29.5	22.2% (3.4)
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N is more significant contributor to media cost and energy.

Cost and energy saving related to media N & P use compared to Redfield media

Per tonne DW	Cost savings (%)	Energy saving (%)
Ratio increase	72.8	69.9
ADE use	98.5	99.0

Input of P equivalent to only 0.2% of biomass energy content

Conclusions

- Consideration of nutrient requirements not always accurate in literature
- Lipid content significantly affects system requirements
- Increase of media N:P ratio reduced P requirement by > 50%, reduces N + P cost and energy input by ~**70%**
- Use of ADE nutrients resulted in total N replacement and >70% of P (depending on N:P ratio)
- ADE reduces N + P cost and energy input by **99%**

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THANK YOU!
ANY QUESTIONS?

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Optimising P-usage – Waste nutrients

Growth parameters of *Nannochloropsis* sp. grown on media with different percentages of N replaced with ADE (n = 3, mean + 1 SD).

	Control	25% N	50% N	100% N
Exp. Growth rate (d^{-1})	0.53 ± 0.05	0.52 ± 0.06	0.53 ± 0.04	0.51 ± 0.03
Max. DW Prod. (mg L^{-1} d^{-1})	56.2 ± 2.2	57.6 ± 2.6	56.2 ± 1.4	54.5 ± 3.8
Max. lipid content (% DW)	51.9 ± 1.2	51.7 ± 0.8	48.0 ± 2.3	49.8 ± 1.1
N content (% DW)	2.6 ± 0.2	2.6 ± 0.1	3.0 ± 0.3	2.7 ± 0.2
P content (% DW)	0.40 ± 0.02 ^{a}	0.34 ± 0.03 ^{ab}	0.36 ± 0.02 ^{ab}	0.38 ± 0.04 ^{c}

Significantly different treatments are represented by different letters (One-way ANOVA with Tukey post hoc, $p < 0.05$).