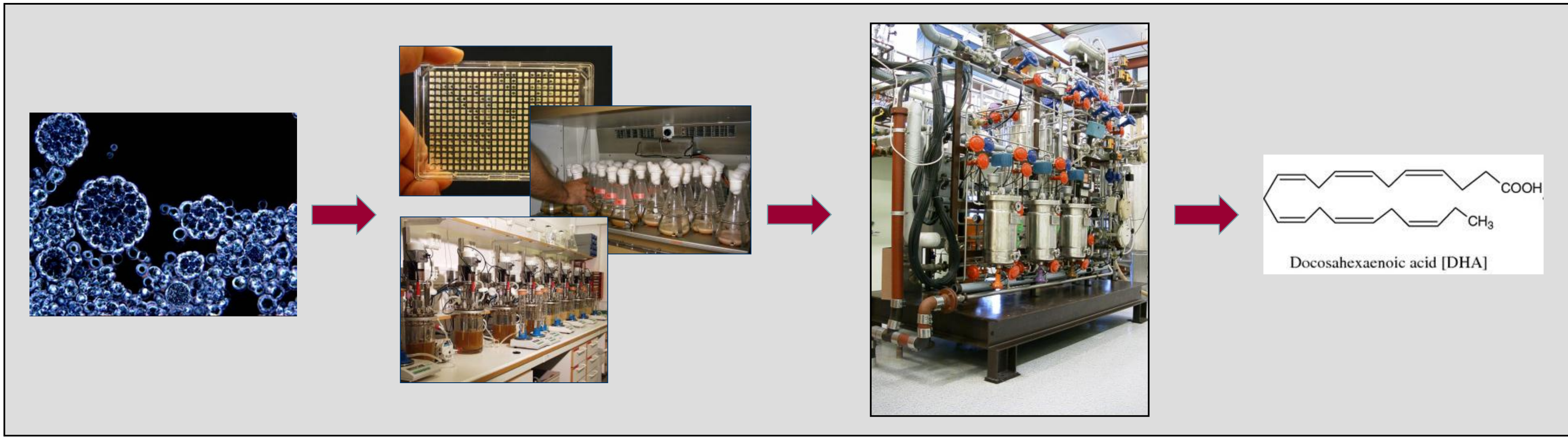
PRODUCTION OF DHA BY HETEROTROPHIC MICROORGANISMS



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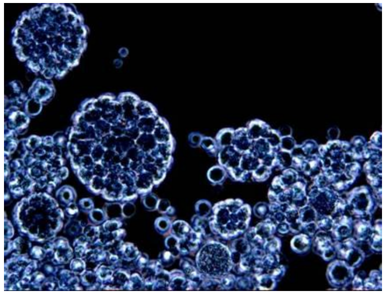
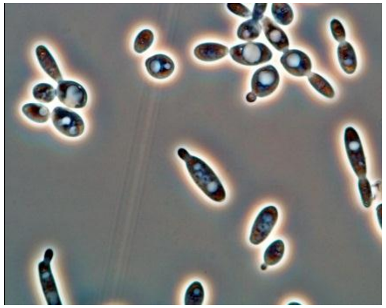
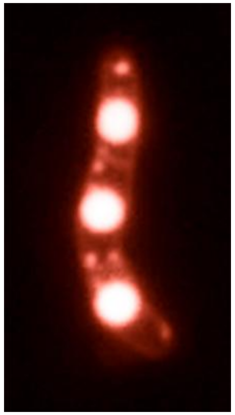
Heterotrophic ω3-PUFA producing organisms

- **Organisms**
  - Lipid accumulating:
    - Microalgae
    - Thraustochytrids
    - Fungi and yeast
    - Bacteria
  - Lipid accumulating **and** EPA/DHA producers:
    - Marine microalgae and thraustochytrids
    - Genetically engineered yeast
- **Carbon sources**
  - Typically glucose or sucrose







Physiology of lipid and EPA/DHA-production

- **Location of EPA/DHA in significant amounts:**
  - Membranes (phospho- and glycolipids):
    - Many marine, heterotrophic species, but membrane lipids <10 % of dw
    - Photosynthetic algae (chloroplast membranes)
  - Storage lipids (triacylglycerols):
    - Only *Crypthecodinium cohnii* and species of thraustochytrids
    - Accumulation is induced by nutrient limitation and energy in excess

Location of EPA / DHA (high levels, e.g. >20 % of TFA)	Organisms	Lipid content [% of dw]
Membrane lipids (glyco- and phospholipids)	Marine bacteria	<10
	Thraustochytrids	<10
	Heterotrophic microalgae	?
	Phototrophic microalgae	15 - ?
Storage lipids (triacylglycerol)	Thraustochytrids	> 50 (40-70)
	Heterotrophic microalgae	>50

Productivities

Heterotrophic organisms	Cell density [g dw/l]	DHA			Reference
		[% of dw]	[g/l]	[g/l·d]	
C. cohnii	109	17	19	1.2	De Swaaf et al., 2003
thraustochytrid strain 12B	21	27	5.6	2.8	Perveen et al., 2006
S. limacinum SR21	59	26	15.5	3.0	Yaguchi et al., 1997
S. limacinum SR21	62	32	20	2.9	Huang et al., 2012
Aurantiochytrium sp.	90-100	15	14	2.2	Jakobsen et al., 2008
Schizochytrium sp.	160-180	25	40-45	10-12	US 7732170

Phototropic algae	1-4	3-5	0.05-0.2	≤ 0.06

Production technology

- **Fermentation**
  - Well-established large scale technology, with an annual production of:
    - 2.5-3 mill. tonnes amino acids (lysine and glutamate)
    - 1.5-2 mill. tonnes citric acid
    - Production plants comprising >10 reactors of 300-500 m^3

Required capacities for feed applications

25 000 tonne EPA/DHA (100 000 tonnes "fish oil equivalents")

Productivity [g/l·d]	Reactor volume [m^3]	Plant size	Technology status	Ground area
10	6 850	15-20 reactors á 350-450 m^3	Existing	~1000 m^2
0.06	1 141 550	Pond: 6300 ha* Tubular: 2550 ha* Flat panel: 1980 ha*	Need to be developed	20-60 km^2

*: Ground area; ratios (m^3/ha) from Norsker et al. (2011)

Costs

- Calculations based on cost analyses and information on large scale fermentation processes for amino acids
- A "value production" of 125-250 €/m^3,day is required for an economical feasible process (15 % internal rate of return)

Required selling price for DHA as a function of productivity for 150 €/m^3,d "value production"

Productivity [g/l·d]	Selling price [€/kg]			
	DHA	Oil		
		40 %*	25 %*	10 %*
5	30	12.00	7.50	3.00
10	**15**	**6.00**	**3.75**	**1.50**
15	10	4.00	**2.50**	**1.00**
25	6	2.40	1.50	0.60

*: % DHA of TFA

Fish oil: ~ 1 €/kg, corresponding to 4 €/kg EPA/DHA (or 10 €/kg DHA)

Phototrophic: 3 €/kg dw (Norsker et al., 2011).

If 5 % EPA-> Production costs 60 €/kg, selling price (+50 %) 90 €/kg

Optimization and cost reduction

Potential improvements

- Increased product concentration
  - Cell density
  - Lipid content
  - DHA content of TFA
- Increased growth and production rates

	Cell density [g/l]	TFA [% of dw]	DHA [% of TFA]	Ferm time [h]	DHA productivity [g/l·d]
Current reported	160-180	60-65	40-45	80-90	10-12
Maximum reported for each parameter	180	65	67	80	24
Assumed max for each parameter	200	70	70	70	34

Sustainability

- **Carbon sources**
  - A production of 100 000 t "fish oil equivalents" (25 000 t EPA/DHA) will represent ~2 % of the current global fermentation production based on glucose (starch) and sucrose.
  - Wastes, such as glycerol, can be utilized
  - Lignocellulose and seaweed biorefineries can represent a future carbon source
- **Other issues that have to be considered**
  - Energy
  - Water
  - Other nutrients
  - etc.

Conclusions

- **Heterotrophic production**
  - Technology well established
  - Costs
    - Maximum reported productivities correspond to selling prices of 15 €/kg DHA (several reports -> ~30 €/kg)
    - Process optimization (reduce time, increase lipid and DHA-fraction) => <10 €/kg DHA
  - In a foreseeable future:
    Probably the only realistic technology for production of the required amounts to supply the aquaculture industry

But:

- A systematic comparison of the technologies on the same basis (e.g. production volumes) should be carried out:
  - Costs
    - Need for genetic engineering to improve productivity; realistic achievements with and without
    - For phototropic production:
      - Technology development (increased photosynthetic efficiency)
      - Location
      - Etc.
  - Sustainability (carbon sources, other nutrients, energy, water, location etc.)
  - Product properties (e.g. polar lipids vs triacylglycerol), other valuable compounds (e.g. pigments) etc.

… and, phototropic production may be more competitive for smaller volumes (and other products)!?