Low-cost Production of Single Cell Protein containing Omega-3 Lipids

Alex Ablaev
NanoTaiga
Moscow, Russia
info@nanotaiga.ru

Edward B. Hamrick
Hamrick Engineering
Miami, FL USA
info@cellofuel.com

Agenda

- History of Foam Fermenters
- Making Torula Yeast with Omega-3 Lipids
- Current Market for Torula Yeast (Lallemand)
- Importance of Omega-3 Lipids
- Why are Americans Fat?
- Healthy and Unhealthy Fats
- Global Sources of Carbohydrates
- What's New in CelloFuel Fermenters?
- Contamination Control
- Patent Status
- Project Status

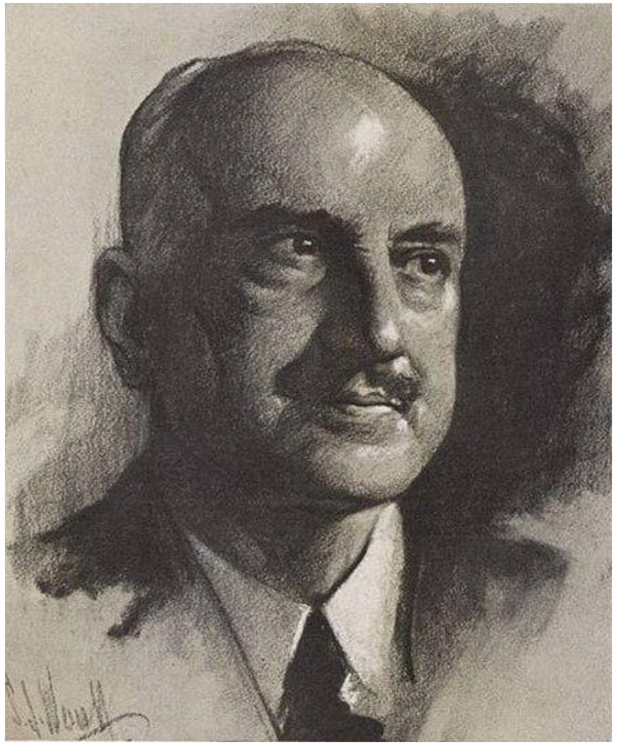
History of Foam Fermenters

George Santayana

Those who cannot remember the past are condemned to repeat it.

Джордж Сантаяна

Те, кто не помнит прошлого, обречены повторять его.



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How is “Zewa” related to the most important invention for Single Cell Protein in the 20th century?

Zellstofffabrik Waldhof

Вальдхофский

целлюлозный завод

Pulp mill Waldhof

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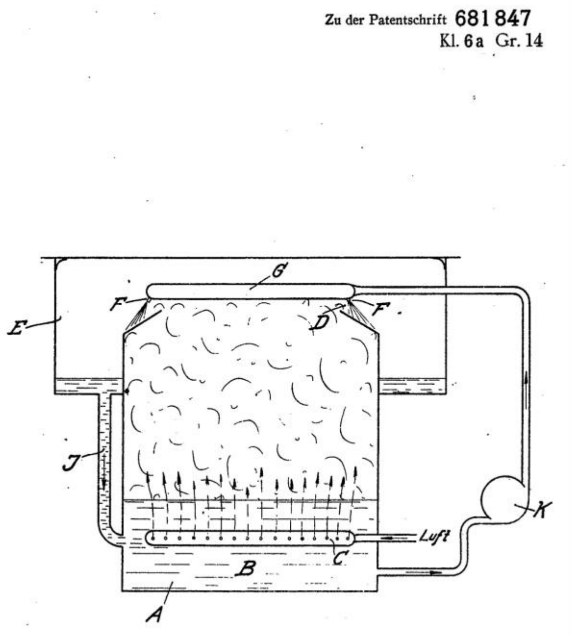
Germany, 1937-1945

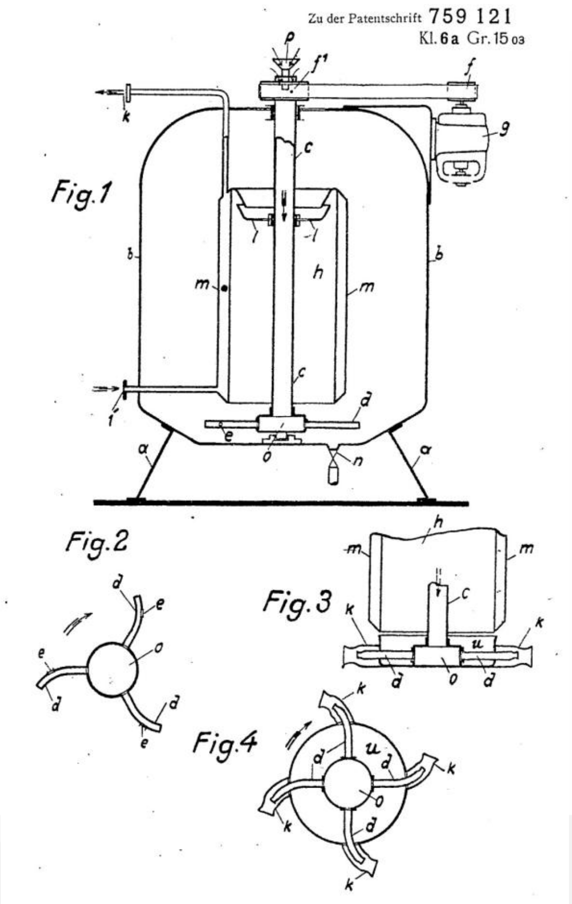
- Germany had a shortage of fuel, protein and fats during WW-II
- German paper mills had a lot of excess sugars as a byproduct of the sulfite process
- Making paper from softwood (spruce) had excess C6 sugars (glucose/mannose), and anaerobic fermentation made ethanol for fuel
- Making paper from hardwood (birch) had excess C5 sugars (xylose) which couldn't be fermented, so was used to make protein from Candida utilis.
- Fermenters made too much foam, and there was little fat in Germany for foam breaking, so a foam fermenter was invented at Zellstofffabrik Waldhof in 1939, producing tens of thousands of tons of protein, eventually in many locations in Germany
- In the 1950's, Waldhof switched to burning excess spent sulfite liquor, protein was no longer needed and was not profitable.
- Waldhof fermenters were never used again in Germany, but you can buy them today in China on Alibaba.com

Previous Solutions – Foam Fermenters

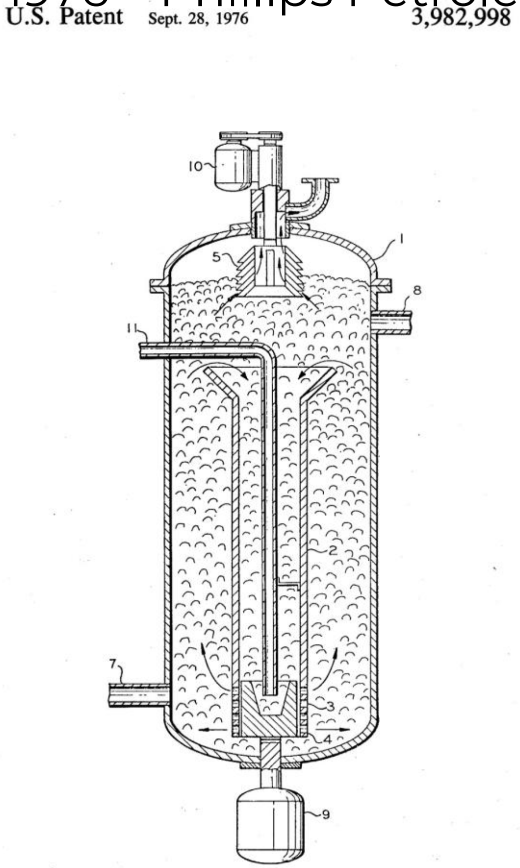
1937 – Stob Fermenter



1940 – Waldhof Fermenter



1976 – Phillips Petroleum



USA, 1945-1965, Foam Fermenters

- Waldhof is near Mannheim, which was in the American sector after the war, and American engineers interviewed Waldhof engineers and made copies of all the drawings for this fermenter.
- Waldhof fermenters were built at sulfite mills in Great Lakes area (Rhinelander, Wisconsin), most wood was hardwood
- Wasn’t very profitable, but cleaned up water from sulfite mills
- Eventually most sulfite mills closed, Kraft pulp mills became dominant, only one Waldhof fermenter remained (Rhinelander).

- In 1947, the long-time director of Waldhof, E. Schmidt, published a complete technical description of the experiences at Waldhof, doubtful that anyone in USA ever read it.
- Schmidt, E. "Eiweiß-und Fettgewinnung über Hefe aus Sulfitablauge." *Angewandte Chemie* 59.1 (1947): 16-20.
- Шмидт Э. «Производство белка и жира дрожжами из сульфитной жидкости». Прикладная химия 59.1 (1947): 16-20.
- “the sum of fat and protein in Torula, around 50%, i.e. if you have 10% fat, there are also 40% protein, but you have 25% fat, so only 25% protein, etc.”

Making Torula Yeast with Omega-3 Lipids

- Growing Candida utilis in high concentrations of glucose accumulates fatty acids
- About 5% glucose accumulates about 7% fatty acids
- Waldhof fermenter can produce 150 g/L yeast dry mass
- This is probably how Schmidt produced 25% lipids in Candida utilis
- Babij, T., F. J. Moss, and B. J. Ralph. "Effects of oxygen and glucose levels on lipid composition of yeast Candida utilis grown in continuous culture." *Biotechnology and bioengineering* 11.4 (1969): 593-603.

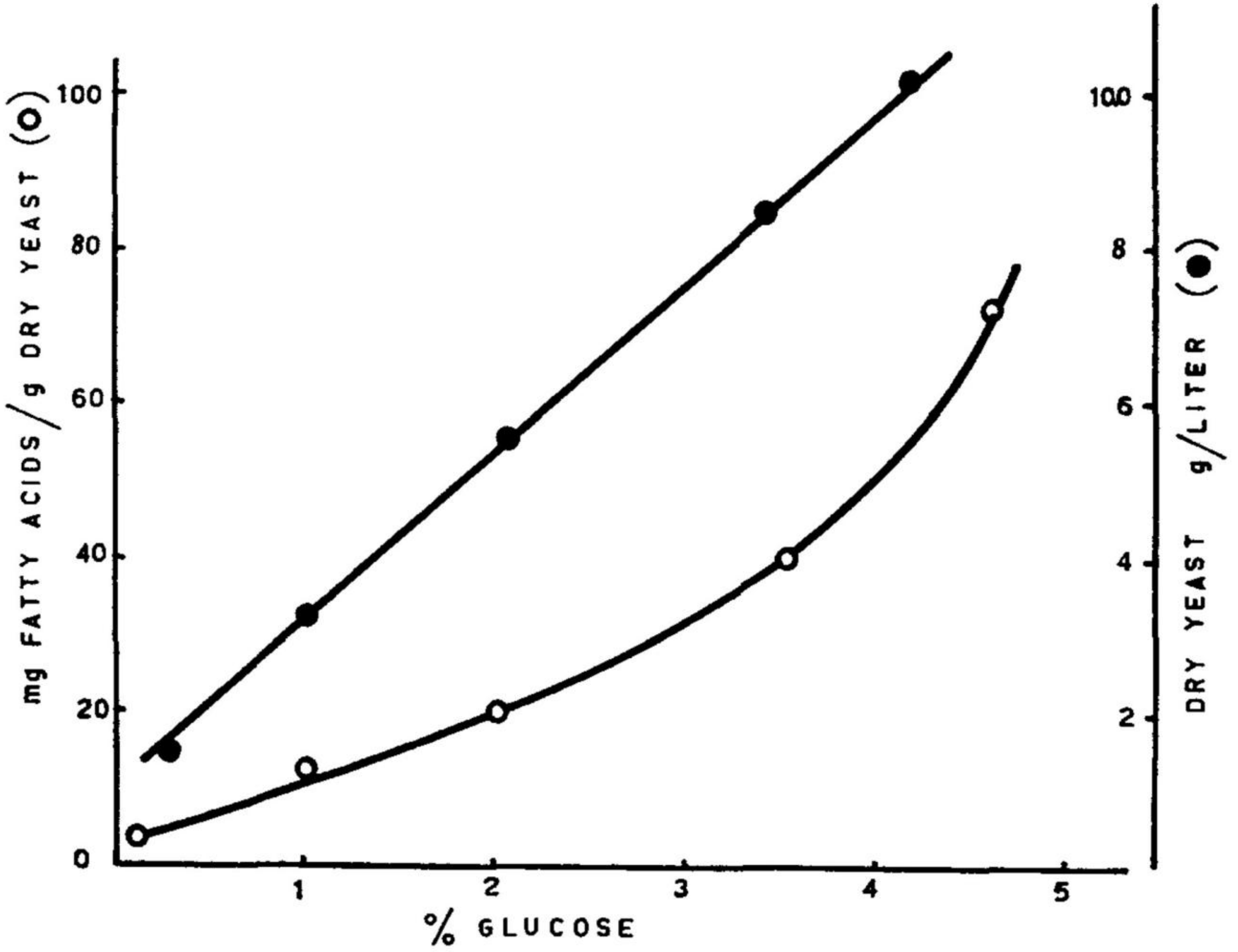


Fig. 3. Changes in lipid content of yeast cells with increasing glucose concentration.

Making Torula Yeast with Omega-3 Lipids

- Higher oxygen concentration leads to higher linonenic acid (C18:3, Omega-3) concentration
- Can produce up to 240 uM oxygen concentration in foam fermenter
- This shows that a foam fermenter with Candida utilis can produce lipids with 35% Omega-6 and 30% Omega-3
- Babij results confirmed in Johnson, B., Sheila J. Nelson, and C. M. Brown. "Influence of glucose concentration on the physiology and lipid composition of some yeasts." *Antonie van Leeuwenhoek* 38 (1972): 129-136.

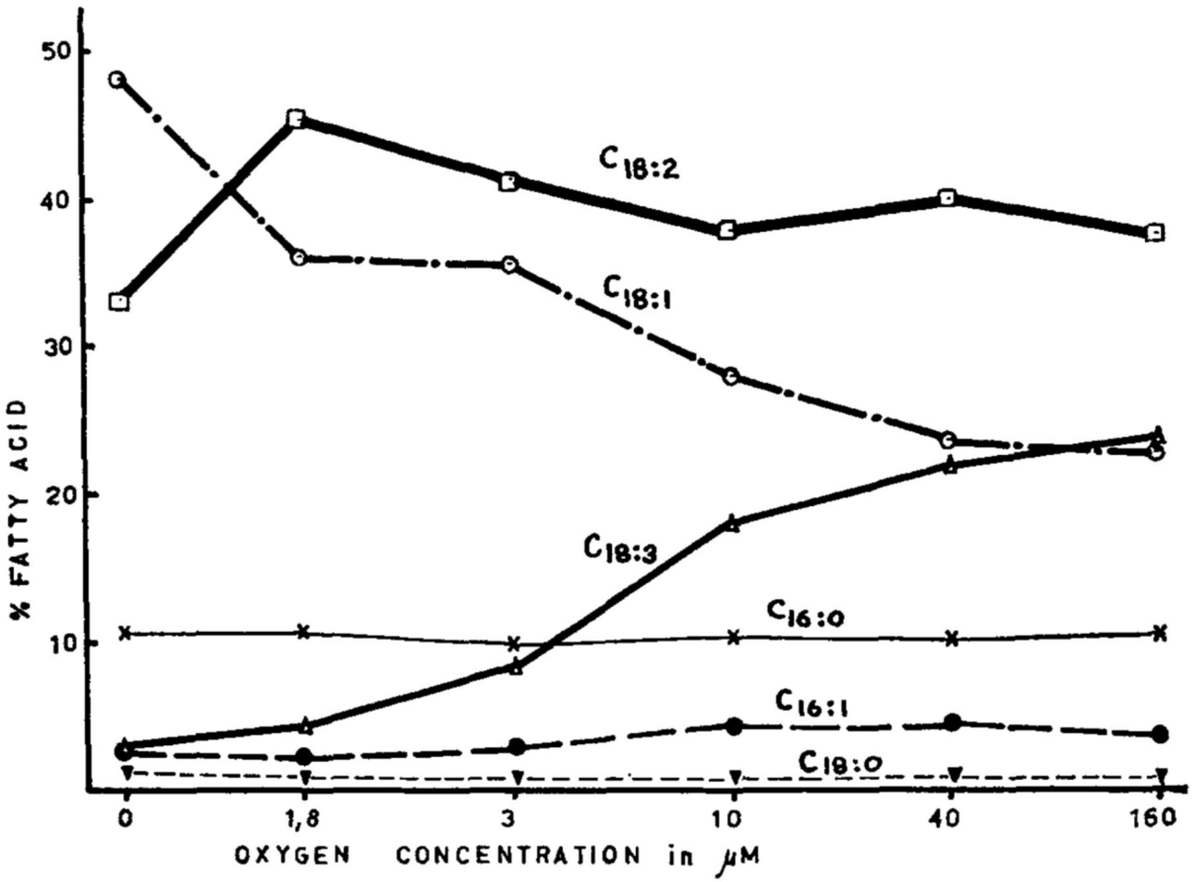


Fig. 2. Effects of oxygen tensions on fatty acid composition expressed as % of total fatty acids at high glucose concentration.

Key: $C_{16:0}$ Palmitic; $C_{16:1}$ Palmitoleic; $C_{18:0}$ Stearic; $C_{18:1}$ Oleic; $C_{18:2}$ Linoleic; $C_{18:3}$ Linolenic.

Current market for Torula yeast (Lallemand)

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- Lallemand is now the world leader in producing Candida utilis (Torula) for the food industry, in Rhinelander, Wisconsin
- Purchased Waldhof fermenter and assets of Provesta (Phillips Petroleum fermenter)
- Sulfite pulp mill is closed, Lallemand uses glucose and molasses, standard batch aseptic aerobic fermenter, doesn't use Waldhof fermenter any more.
- Lallemand Torula has no Omega-3 lipids
- There's something magic about a Waldhof fermenter that produces Torula yeast with Omega-3 lipids

Lake States®
Classic Torula yeast

LAKE STATES®

Primary grown, dried whole cell torula inactive yeast. It is a specialty product range with the unique ability of delivering superb flavor carrier properties in different food applications, with distinctive savory notes and superior texture enhancement.

GLUTEN FREE | Clean Label | VEGAN | GMO FREE | HALAL CERTIFIED | KOSHER CERTIFIED

DESCRIPTION

Lake States® production begun in 1948 in Rhinelander, Wisconsin. For over 50 years the site has been a leader in the development and commercialization of inactive dried torula yeast for the food industry, pharmaceuticals, industrial fermentations, animal feed, and pet food markets. Thanks to Lallemand investments, it is today the largest torula inactive yeast production site in the world.

The Lake States® products can be used to help to reduce the use of additives such as MSG and HVP. They are designed to impart savory notes, enhance texture and nutritional characteristics in foods. They are ideal for a broad range of processing temperatures and preparation techniques, including microwave.

If you are interested in receiving "Lake-States®" leaflet, or to learn more about our inactive yeast range, please contact us or send an e-mail to info@bio-lallemand.com.

We will be happy to support you in the choice of the most suitable product!

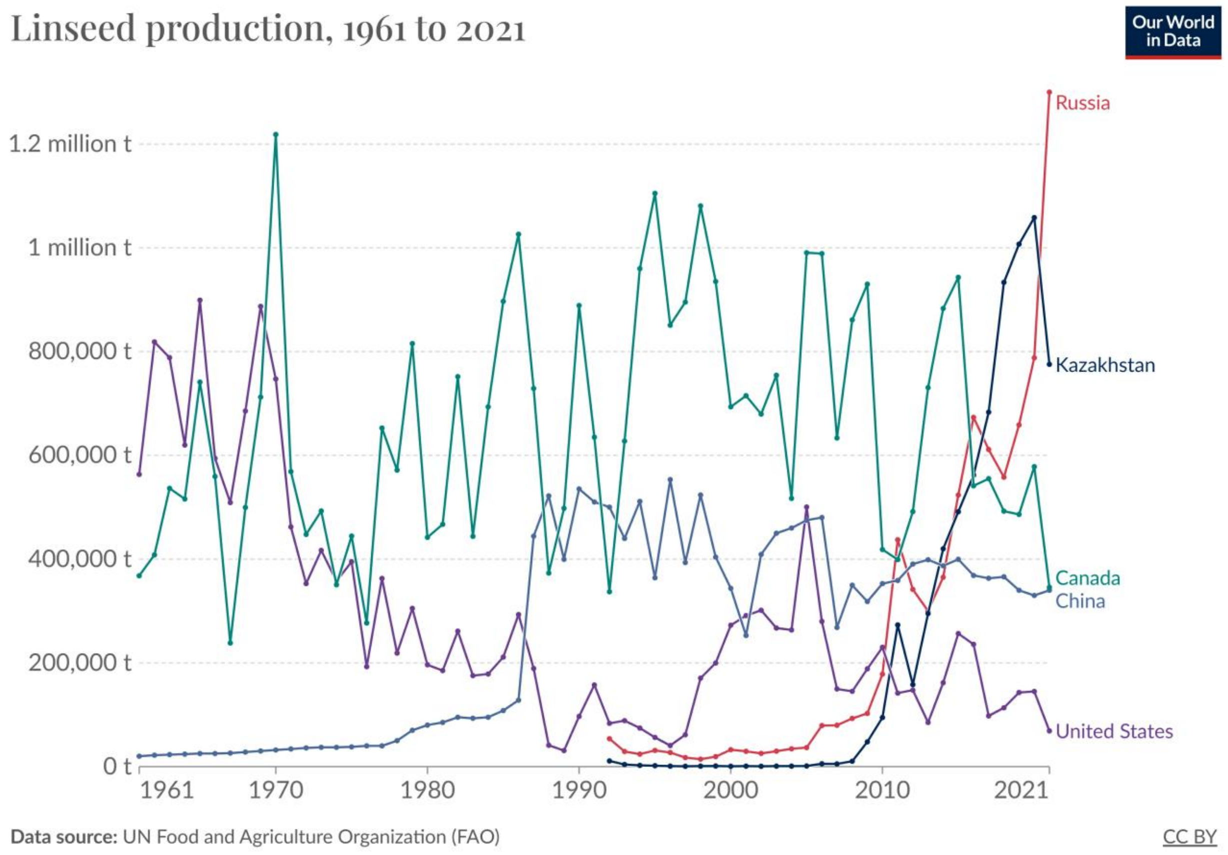
PRODUCT HIGHLIGHTS

RECIPES

Why do companies sell eggs containing “Omega-3”?

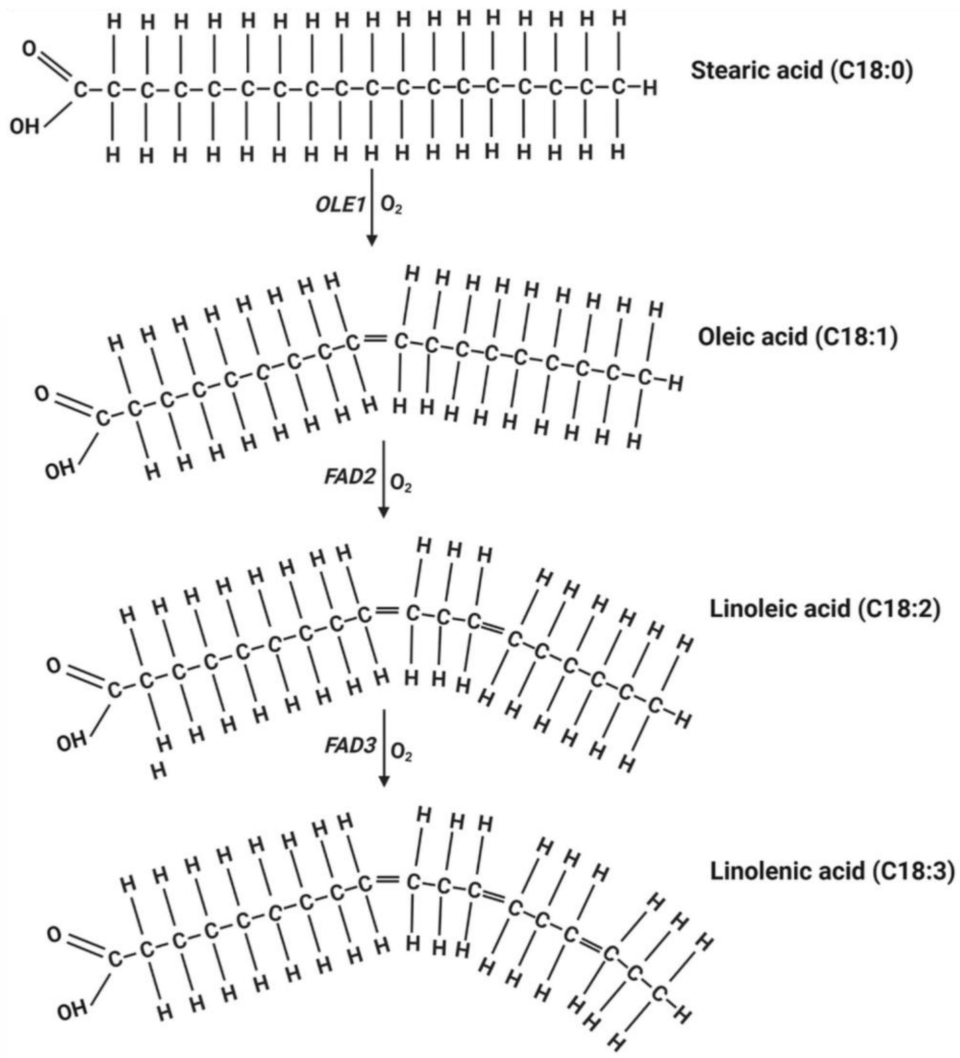
Why are Russia and Kazakhstan the biggest producers of Linseed?

- Turin, E. N., et al. "Linum usitatisimum L. is the most important crop in Russia for the production of high-quality oil with low cost." IOP Conference Series: Earth and Environmental Science. Vol. 640. No. 4. IOP Publishing, 2021.



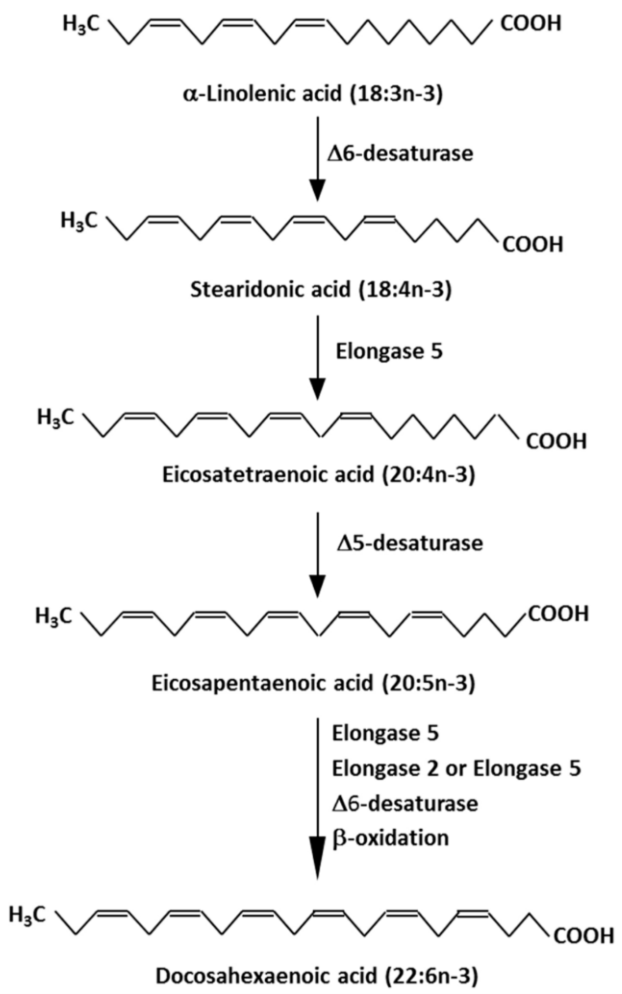
Importance of Omega-3 Lipids

- Omega-3 and Omega-6 fatty acids are essential to human life
- The human body does not have FAD2 and FAD3 enzymes so we need linoleic acid (Omega-6) and linolenic acid (Omega-3) in our diet
- Ratio of Omega-6 to Omega-3 must be between 2:1 and 1:2 for optimal health
- More oxygen = more Omega-3
- The Waldhof fermenter has higher dissolved oxygen at high cell concentrations than any other type of fermenter



Importance of Omega-3 Lipids

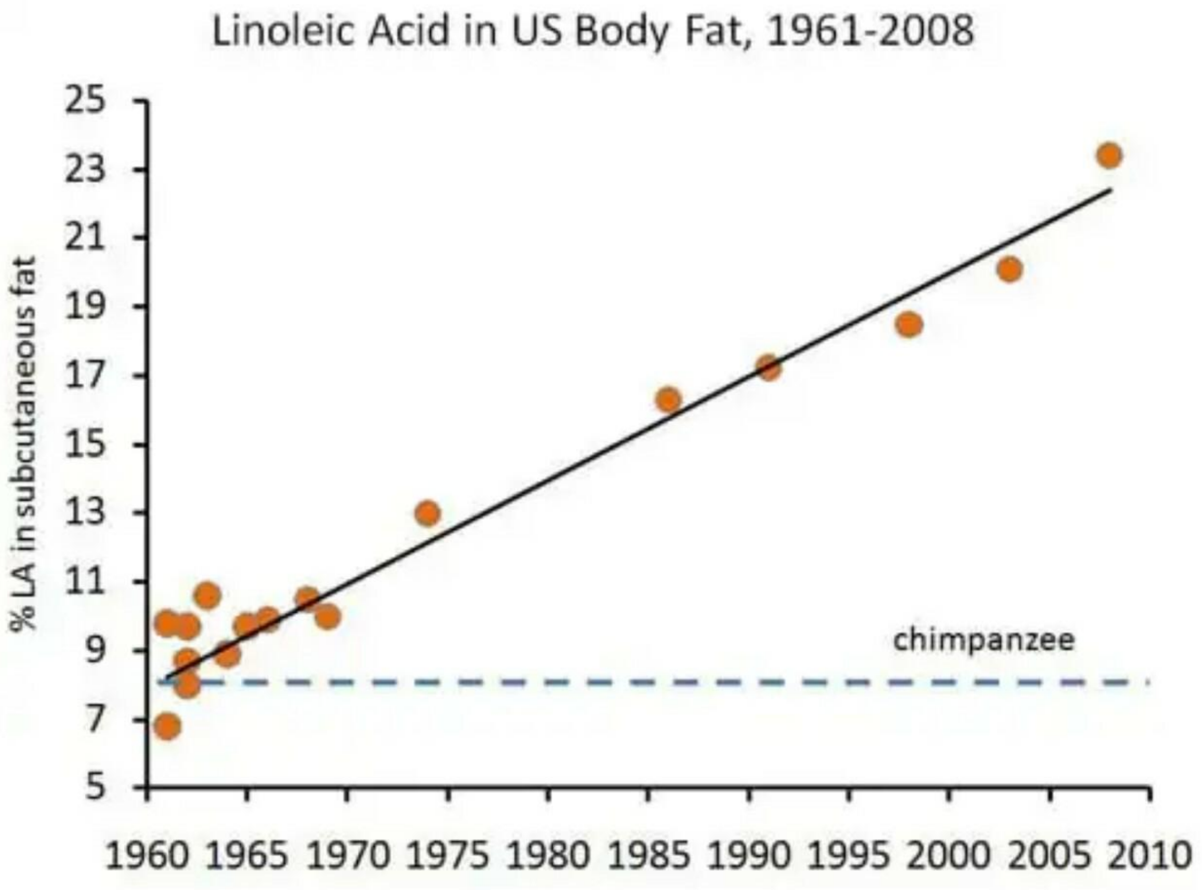
- Omega-3 is precursor to EPA and DHA, the major fatty acid in the brain is DHA
- The human body can produce EPA and DHA, but the more Omega-6, the less EPA and DHA (competition for the same enzymes)
- Chickens and salmon (and most animals) can produce EPA and DHA from Omega-3
- Epidemiological studies suggest that consumption of DHA is associated with a reduced incidence of Alzheimer disease. Animal studies demonstrate that oral intake of DHA reduces Alzheimer-like brain pathology.



Why are Americans Fat?

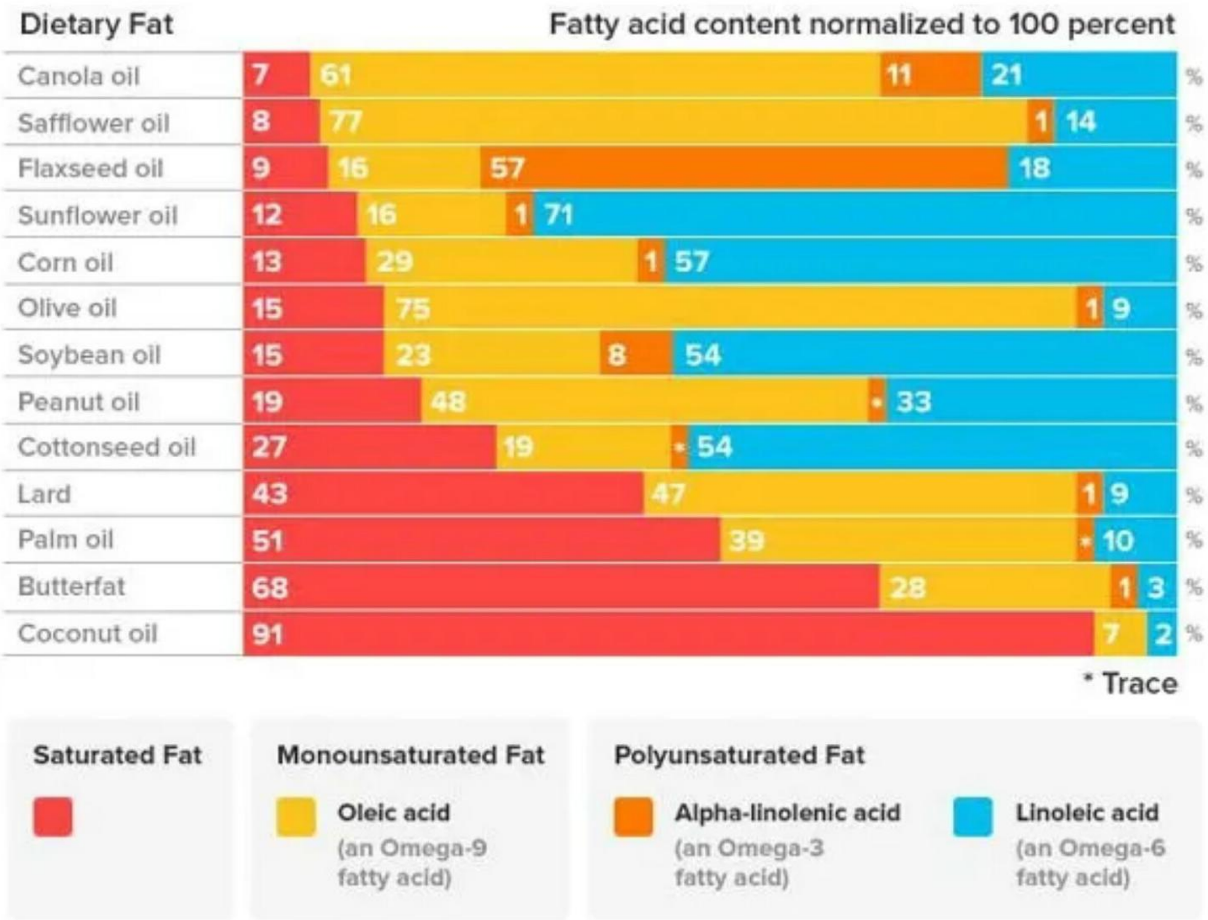
- America's most widely consumed oil causes genetic changes in the brain. New UC Riverside research shows soybean oil not only leads to obesity and diabetes, but could also affect neurological conditions like autism, Alzheimer's disease, anxiety, and depression.

  University of California, Riverside, Jan 17, 2020
- Soybean oil has a 7:1 ratio of Omega-6 linoleic acid to Omega-3 linolenic acid.
- A ratio of more than 2:1 Omega-6 to Omega-3 leads to reduced levels of DHA in the body. DHA is the main fatty acid in the brain.
- Animals are usually fed grain-based feeds containing soy and corn. Fats in the meat are mostly Omega-6 while grass fed animals are 1:1.



Healthy and Unhealthy Fats

- Unhealthy fats have a ratio of linoleic acid to linolenic acid greater than 2:1. This leads to low production of DHA, which is needed for the brain.
- Soybean (7:1), corn (57:1), sunflower (71:1) and cottonseed oil (54:1) are very unhealthy
- Canola (rapeseed) oil (2:1) is reasonably healthy, most margerine in the world today uses this
- Flaxseed (linseed) oil (1:3) is the healthiest, but oxidizes easily so not useful for cooking oil and margarine
- Can feed flaxseed to chickens without grinding, commonly used to make Omega-3 eggs



Global Sources of Carbohydrates

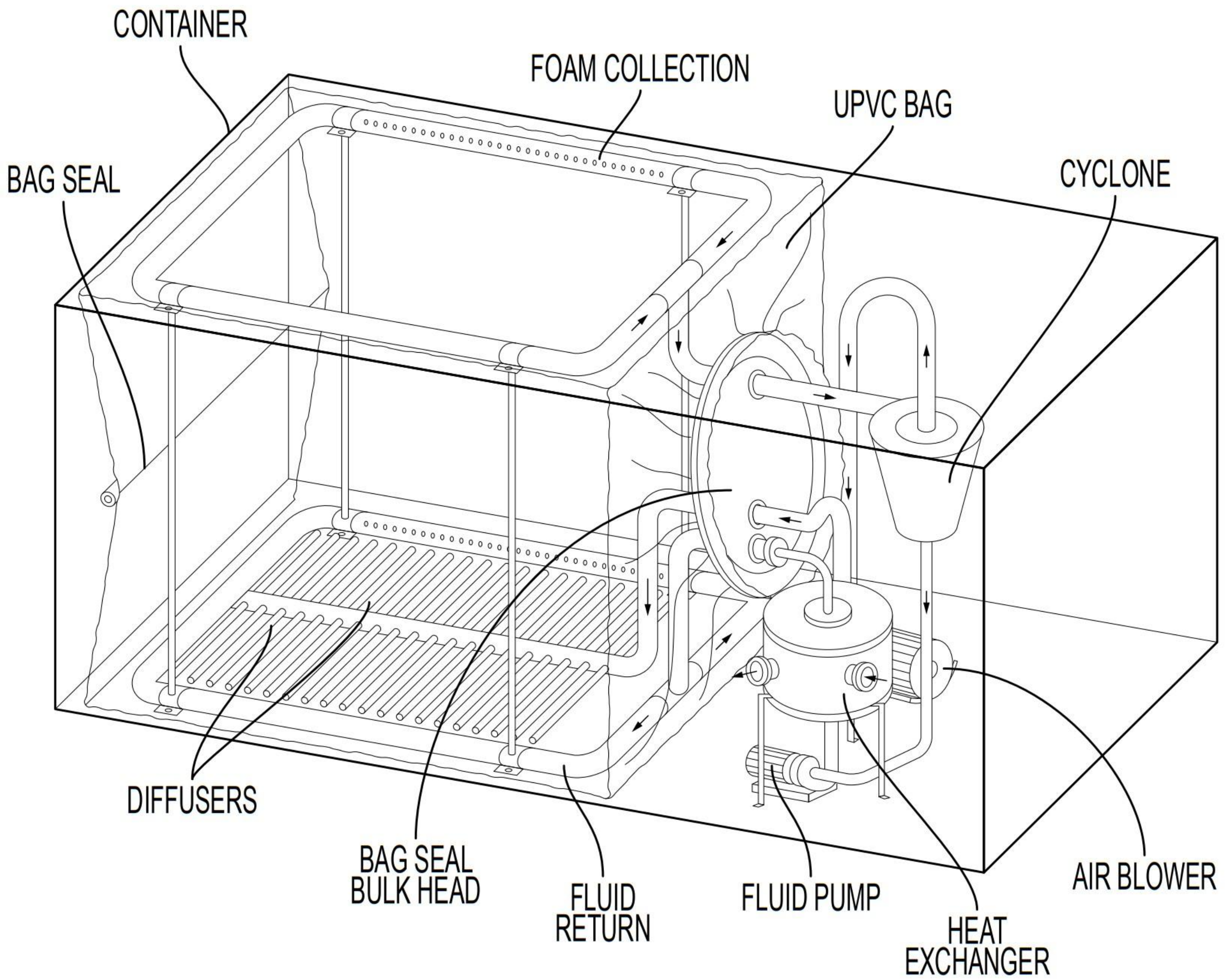
- Russia produces more than 100 Mt of grain, USA produces 400 Mt of corn, 40% for fuel ethanol, China is largest agricultural producer.
- Brazil, India, Russia, USA and EU produce a lot of sugar from sugarcane and sugar beet
- Main carbohydrate in Africa is cassava, which is very low in protein and lipids. Foam fermenter can make protein and lipids from Cassava.
- More than 90% of all new cars will be Electric Vehicles by 2030, so demand for bioethanol will drop significantly by 2030, so lots of excess corn, wheat and sugar

- What can all these excess carbohydrates be used for?
- Single Cell Protein for animal feed? Fish feed doesn't need carbohydrates, so Candida utilis with Omega-3 lipids is best to displace fish meal and fish oil.
- Beyond Meat – protein from peas, mung beans, faba beans, brown rice doesn't taste as good as salmon and chicken.
- People don't want to eat yeast but love to eat salmon and chicken. Use Omega-3 Candida utilis to feed fish and chickens, thus making fish and chicken with EPA and DHA.

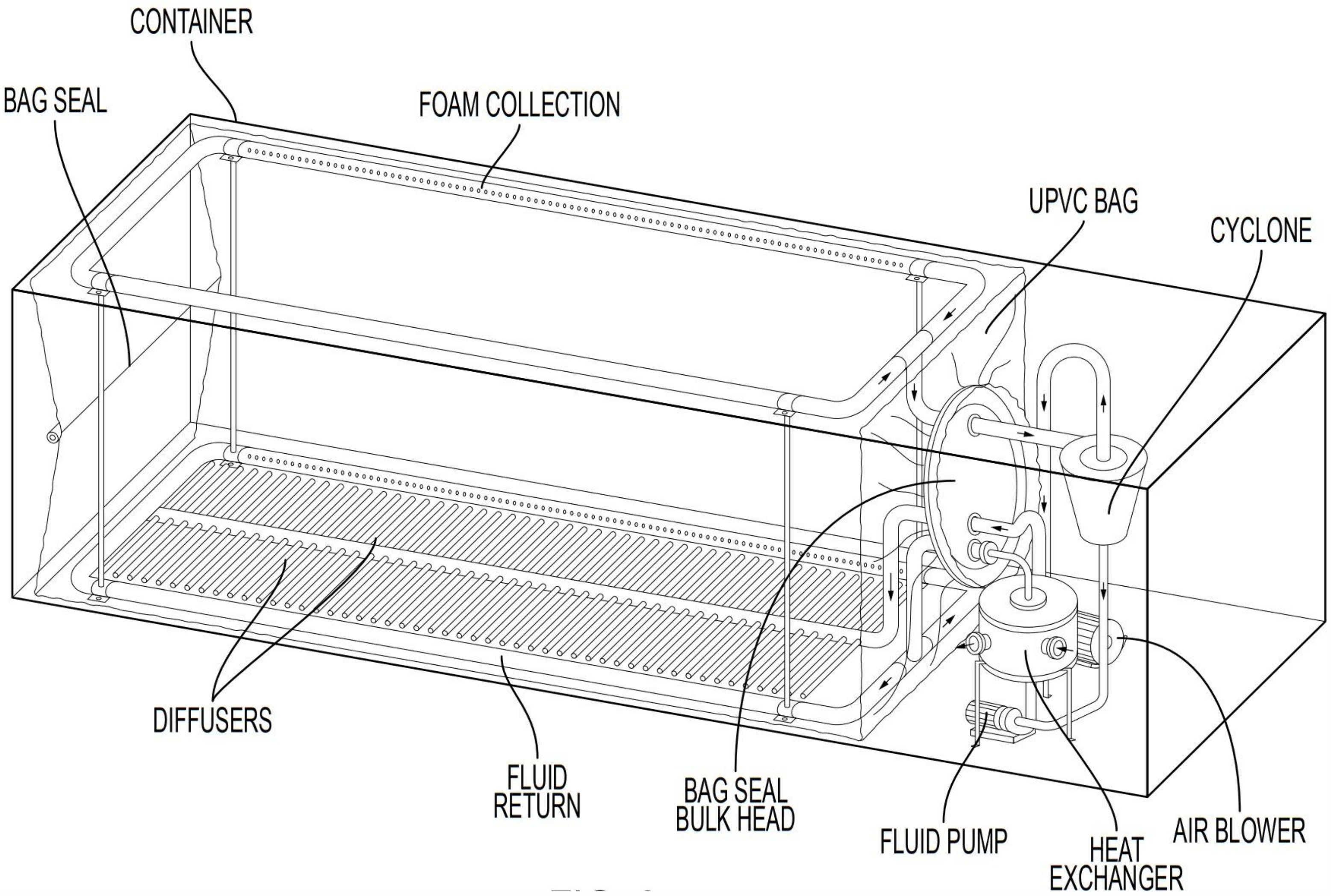
What's New in CelloFuel Fermenters?

- Waldhof fermenter has a 1.5 m diameter spinning disc to make a 10% emulsion, Cellofuel fermenter uses 75mm bubbler tubes to make foam with 10% liquid, using much less energy with no moving parts.
- Waldhof fermenter removes heat by cooling emulsion with heat exchanger, but emulsion is an insulator and can't easily be cooled. Cellofuel fermenter cools fermentation liquid after breaking foam with cyclone.
- Waldhof fermenter uses spinning disk to break foam and reinject air. CelloFuel fermenter uses cyclone to break foam and reinject air, which uses much less energy with no moving parts.
- Waldhof fermenter uses a stainless steel fermenter, Cellofuel fermenter uses a UPVC fermenter in a shipping container, reducing cost.
- Waldhof fermenter uses stainless steel heat exchanger, Cellofuel fermenter uses either titanium or 316L heat exchanger to reduce nickel leaching, which works with contamination control (discussed later).

20 ft. CelloFuel Container



40 ft. CelloFuel Container



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Modeled 4 cases:

1) Candida utilis, ¼ size, 10% biomass
2) Candida utilis, full size, 10% biomass
3) Methylococcus capsulatus, full size, 10% biomass
4) Methylococcus capsulatus, full size, 5% biomass

1	Microorganism and nitrogen source	Candida utilis + urea	Candida utilis + urea	Methylococcus capsulatus + KNO3	Methylococcus capsulatus + N2
2					
3	Doubling time (min)	105	105	120	140
4	Heat (MJ/mol_O2)	0.40	0.40	0.47	0.47
5	Fermentation temperature (C)	30	30	45	45
6					
7	Substrate (sub)	CH2O	CH2O	CH4	CH4
8	Molar mass of substrate (g/mol_sub)	30	30	16	16
9	Cell composition (cell)	C5H10O2.5N	C5H10O2.5N	C5H9O2N	C5H9O2N
10	Molar mass of cells (g/mol_cell)	124	124	115	115
11	Molar yield from substrate (mol_cell/mol_sub)	0.13	0.13	0.13	0.13
12	Mass yield from substrate (g_cell/g_sub)	0.55	0.55	0.92	0.92
13	Cell yield from O2 (mol_cell / mol_O2)	0.40	0.40	0.11	0.11
14	Ratio of substrate to O2 (mol_sub/mol_O2)	3.03	3.03	0.83	0.83
15					
16	Cell yield from O2 (g_cell/mol_O2)	50.09	50.09	12.27	12.27
17	Oxygen concentration (mol_O2/mol_gas)	0.21	0.21	0.21	0.08
18					
19	Fermenter width (m)	0.60	2.40	2.40	2.40
20	Fermenter height (m)	0.60	2.40	2.40	2.40
21	Fermenter length (m)	4.00	4.00	4.00	4.00
22	Liquid height (m)	0.15	0.15	0.15	0.15
23	Fermenter foam volume (m3)	1.08	21.60	21.60	21.60
24	Plastic tube roll-flat width (m)	1.20	4.80	4.80	4.80
25	Plastic tube diameter (m)	0.76	3.06	3.06	3.06
26					
27	Cell density in liquid (g_cell/g_liquid)	0.10	0.10	0.10	0.05
28	Liquid fraction (fraction)	0.10	0.10	0.10	0.10
29	Fermenter liquid (kg)	108.00	2,160.00	2,160.00	2,160.00
30	Fermenter gas (L)	972.00	19,440.00	19,440.00	19,440.00
31	Fermenter cells (kg_cell)	10.80	216.00	216.00	108.00
32	Fermenter gas (mol_gas)	39.07	781.46	744.62	744.62
33	Fermenter O2 (mol_O2)	8.18	163.68	155.97	59.57
34					
35	Cell mass grown if all O2 used (kg)	0.41	8.20	1.91	0.73
36	Time to use all O2 (min)	5.64	5.64	1.53	1.36
37	Fan airflow to use all O2 (m3/h)	10.33	206.68	764.01	856.42
38					
39	Diameter of cyclone inlet (m)	0.05	0.10	0.10	0.10
40	Number of cyclones	1.00	3.00	4.00	4.00
41	Min inlet velocity (m/s)	7.00	7.00	7.00	7.00
42	Fan airflow to achieve min inlet velocity (m3/h)	49.48	593.76	791.68	791.68
43	Fan airflow (m3/h)	49.48	593.76	791.68	856.42
44					
45	Foam cycle time (min)	1.18	1.96	1.47	1.36
46	Cell mass grown per cycle (kg)	0.08	2.82	1.85	0.73
47	O2 consumed per cycle (mol_O2)	1.68	56.29	150.49	59.57
48	Heat generated per cycle (kJ)	673.69	22,514.75	70,280.53	27,818.88
49	Temperature rise per cycle (C)	1.49	2.49	7.78	3.08
50					
51	Number of 750 mm diffusers needed	6.60	79.17		
52	Number of 1 m diffusers needed		59.38	79.17	85.64
53	Spacing of diffusers (m)		0.10	0.10	0.10
54	Number of diffusers that will fit		78.00	78.00	78.00
55					
56	Fan power needed (kW)	0.41	4.95	6.60	7.14
57					
58	Cooling needed (kW)	9.53	191.02	795.04	340.43
59	Cooling water temperature (C)	15.00	15.00	15.00	15.00
60	Cooling water mass flow (kg/s)	0.15	3.04	6.33	2.71
61	Cooling water volume flow (m3/hr)	0.55	10.95	22.79	9.76
62	Cooling water volume flow (L/min)	9.10	182.53	379.85	162.65
63	Heat exchanger area (m2)	0.24	4.72	9.82	4.20
64					
65	Recirculation slurry flow (m3/h)	5.50	22.96	84.89	95.16
66	Recirculation slurry flow (liters per minute)	91.63	382.75	1,414.84	1,585.95
67	Harvest of cells (kg/h)	4.29	87.17	75.19	32.19
68	Harvest slurry flow (m3/h)	0.04	0.87	0.75	0.64
69	Harvest slurry flow (liters per minute)	0.72	14.53	12.53	10.73
70	Nutrient feed (m3/h)	0.04	0.87	0.75	0.64
71	Nutrient feed)(liters per minute)	0.72	14.53	12.53	10.73
72					
73	Yearly substrate (ton_sub/year)	68.28	1,385.89	715.95	306.52
74	Yearly productivity (ton_cell/year)	37.62	763.59	658.67	282.00
75	Hourly productivity (kg/hr)	4.29	87.17	75.19	32.19
76					
77	Cost of substrate ($/ton_sub)	500.00	500.00	110.00	110.00
78	Cost of nitrogen ($/ton_cell)	46.28	46.28	28.02	-
79	Cost of electricity ($/ton_cell)	184.25	14.28	18.75	45.47
80	Sale of cells ($/ton_cell)	1,500.00	1,500.00	1,500.00	1,500.00
81					
82	Profit/year ($)	14,000.00	406,000.00	878,000.00	376,000.00
83	CAPEX ($)	50,000.00	50,000.00	50,000.00	50,000.00

Contamination Control

- Waldhof fermenter ran for months at a time with an open top with no bacterial contamination. Analysis shows this was due to residual sulfite.
- Bacteria need nickel to grow on urea (needed by urease enzyme). CelloFuel container designed to not leach nickel into liquid.
- Yeasts need biotin to grow on urea without nickel (needed by urea amidolyase enzyme) and no biotin is added during fermentation. Candida utilis is biotin prototrophic (makes it's own biotin) and other contaminating yeasts, like Dekkera bruxellensis, need added biotin
- CelloFuel fermenter grows Candida utilis with urea as the sole nitrogen source, without added biotin and without nickel leaching.
- The fastest growth of Candida utilis is when growing using urea as the nitrogen source.
- Testing of this contamination control technique has been successfully completed and the final patent application will be submitted soon.

Patent Status

Patent pending at U.S. Patent Office,

"AEROBIC FERMENTATION USING PNEUMATIC FOAM"

application number 63/530,954, priority date of 5 August 2023

"CONTAMINATION CONTROL WHEN GROWING YEASTS"

Application number 63/534,123, priority date of 23 August 2023

Planned PCT submission to RU, US, CN, IN, BR, EU, QA

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Project Status

CelloFuel
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CelloFuel
Portable Biomass Refinery

Scale model (1/4 height/width), 600 mm x 600 mm x 4000 mm

Спасибо за внимание!

Задавайте любой вопрос

Alex Ablaev
NanoTaiga
Moscow, Russia
info@nanotaiga.ru

Edward B. Hamrick
Hamrick Engineering
Miami, FL USA
info@cellofuel.com