Highly efficient liquid-liquid lipid extraction from wet microbial cells

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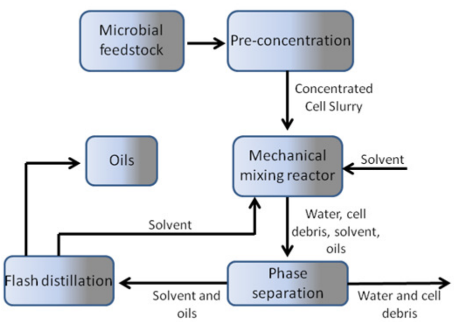
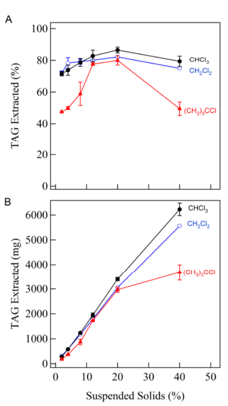
Abstract

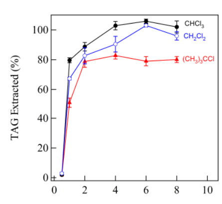
Microbial derived biofuels comprise an important replacement to petroleum based fuels. Unlike traditional biofuel feedstocks, microbial cultivation does not compete directly with agriculture for land and water resources. Despite this benefit many obstacles to economical production of microbial oils remain. One such obstacle is the high cost of harvesting and drying microbes prior to lipid extraction and conversion. Here we report the development of a liquid-liquid extraction technique that efficiently extracts lipids from cell slurries of oleaginous microorganisms containing as little as 2% solids. At the heart of the process is an organic solvent combined with high speed mixing to extract lipids from microbial cells suspended in solution. We used the program ASPEN to screen thousands of potential solvents based on partition coefficients, which were then used, along with cost, health hazard and boiling point to select solvents for initial studies. Several solvents were found to be much more effective than hexane, which is commonly used for lipid extraction. Optimal parameters for extraction of >95% of available triglyceride were determined for the top three solvents, chloroform, methylene chloride, and *tert*-butyl chloride. The process is found to be equally effective at extracting lipids from oleaginous yeast, bacteria, and algae. The energetic cost of the process (13 600 kJ per L of algal oil) is discussed relative to a more traditional approach that requires completely dry microalgae (32 500 kJ per L of algal oil). This process allows for the highly efficient and energetically favorable extraction of wet oleaginous cells.

Results

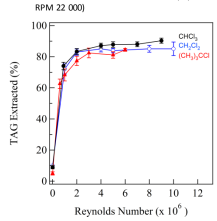




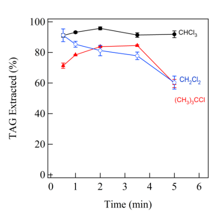
Effect of suspended solids concentration on extraction efficiency. A. The amount of extracted TAG relative to the amount available is shown plotted against the concentration (% w/v) of suspended solids. B. The total TAG extracted from the cells plotted against the concentration of suspended solids (% w/v). Error bars are shown with standard error of the mean (SEM). In each case 30 mL of cell slurry (with varying % solids) was extracted with 60 mL (2:1 solvent:cell slurry) of either chloroform (● $CHCl_3$), methylene chloride (○ CH_2Cl_2), or *tert*-butyl chloride (▲ $(CH_3)_3CCl$) by mixing for 2 min. Reynolds number of 6 x 10^6 for chloroform and methylene chloride and 4 x 10^6 for *tert*-butyl chloride (a lower number had to be used because of equipment limitation, max RPM 22000). The total TAG extractable was determined from the modified Bligh and Dyer extraction (3) performed on dry cell samples.



Determination of the optimal solvent to cell slurry ratio. The amount of TAG extracted relative to the amount available is plotted against a varying solvent:cell slurry ratio. A fixed volume of cell slurry (30 mL, 20% solids) was mixed with varying volumes of chloroform (● $CHCl_3$), methylene chloride (○ CH_2Cl_2), or *tert*-butyl chloride (▲ $(CH_3)_3CCl$) for 2 min and Reynolds numbers for each solvent were 9 x 10^6, 1 x 10^7, and 6 x 10^6 respectively. Error bars are shown with SEM. Reynolds numbers vary between solvents due to differences in density, viscocity, and mechanical limitations of the equipment (max RPM 22 000)



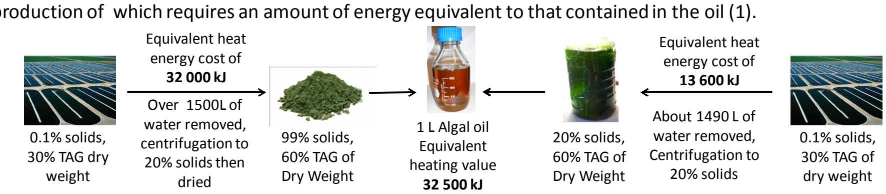
Determination of the optimal Reynolds number for mixing. Chloroform (● $CHCl_3$), methylene chloride (○ CH_2Cl_2), or *tert*-butyl chloride (▲ $(CH_3)_3CCl$) were mixed for 2 min with 30 mL of cell slurry (20% solids) at a 2:1 solvent:cell slurry ratio. The percentage of the total TAG extracted is plotted against the Reynolds number. Error bars are shown with SEM.



Determination of the optimal mixing time. The amount of TAG extracted relative to the total TAG available is plotted against time of mixing. 30 mL of cell slurry (20% solids) is mixed with either chloroform (● $CHCl_3$), methylene chloride (○ CH_2Cl_2), or *tert*-butyl chloride (▲ $(CH_3)_3CCl$) at a 2:1 solvent:cell slurry ratio for varying lengths of time with Reynolds numbers of 9 x 10^6, 1 x 10^7, and 6 x 10^6 respectively. Error bars are shown with SEM. The Reynolds numbers vary between different solvents due to differences in viscocities, densities and mechanical limitations of the equipment used.

Background

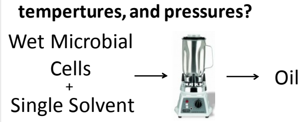
- Traditional extraction of microbial oil requires dry or near dry cell material comprising >99% solids, the production of which requires an amount of energy equivalent to that contained in the oil (1).



- Current extraction methods that utilize <40% solids algal feedstock, known as a wet cell extractions, can accomplish oil extraction with a lower energy input, but require the use of high temperatures and pressures as well as a solvent system involving two or more solvents complicating downstream processing (2).

Background Analysis and Methods

How can liquid-liquid extraction efficiency be increased without the use of a two solvent system and increased tempertures, and pressures?



Find a solvent that can extract efficiently when paired with high mixing speeds.

Solvent	Aspen Rank[a]	Health Hazard[b]	Cost per L[c]	Boiling Point (°C)	Viscocity mPa•s (@20 °C)	Percent TAG Extracted [d]
n-Propylmercaptan	8	2	$74.60	67	0.385	92 ± 1
1-Chlorobutane	17	1	$49.00	77	0.422	90 ± 1
Tert-butyl-chloride	**27**	**0**	**$49.00**	**51**	**0.510**	**87 ± 1**
2-Chlorobutane	29	0	$102.00	68	0.360	87 ± 1
Chloroform	**22**	**2**	**$37.40**	**61**	**0.537**	**86 ± 1**
Methylene chloride	**168**	**2**	**$33.00**	**40**	**0.413**	**82 ± 1**
Dimethyl sulfide	3	2	$42.60	36	0.284	79 ± 1
Diethyl ether	35	2	$48.00	35	0.224	79 ± 1
2-Methyl-1,3-butadiene	12	1	$78.80	34	0.225	78 ± 2
1,1-Dichloroethylene	42	2	$81.00	30	0.840	71 ± 2
Isopropyl chloride	34	1	$91.40	34	0.303	71 ± 3
Allyl Chloride	7	2	$76.80	45	0.354	50 ± 1
Pentane	33	0	$55.50	35	0.224	46 ± 1
n-Hexane	138	2	$36.20	69	0.300	22 ± 1

[a]Solvents were ranked by the relative selectivity obtained by simulating the partitioning of triolein into the solvent from an aqueous solution using Aspen.

[b]Health hazard number for each solvent was obtained from the corresponding material safety data sheet.

[c]The cost per liter of each solvent was obtained from Alfa Aeser catalog prices for 1 L or 500 mL volumes.

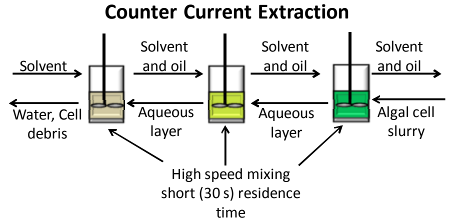
[d]The percentage of the total TAG available recovered by the wet extraction technique for a given solvent. Total TAG content was determined by a modified Bligh and Dyer technique described in the methods section. Wet extraction was carried out by mixing 60 mL of solvent with 30 mL cell slurry (20% solids) mechanically at 20 000 RPM for 2 min.

Conclusions

- Extraction of oil from a cell slurry of oleaginous microrganisms is achieved with greater than 95% efficiency when the combined with an appropriate solvent and high speed mixing.
- This process functions effectively for algae, yeast, and bacteria with high extraction efficiencies.
- The higher the solvent polarity, the higher the extraction efficiency as seen for chloroform, methylene chloride, and tert-butyl chloride.
- The short mixing period required to obtain >95% of the available lipids indicates that this process could be adapted for high-throughput continuous operation

Future Work

Counter Current Extraction



- High efficiency extraction while using non-polar solvents
- High throughput for large scale production

Acknowledgments: US DOE Grant No. DE-EE0003114; USTAR (Utah Science Technology and Research Initiative); Faculty, Staff, Students USU BioEnergy Center

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