Lipid Storage Compounds in Raw Activated Sludge Microorganisms for Biofuels and Oleochemicals Production

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Table S1 Experimental design for preliminary Bligh & Dyer extractions.[a]

Experimental Run	Amount of Solids (g)	% Solids (weight)[c]	Solids to Solvent Ratio (g/L)[d]	Solids to Solvent Ratio (g/L)[e]	Solids to Solvent Ratio (g/L)[f]
1[b]	0.00	0.00	0.00	0.00	0
2	0.10	3.23	5.33	2.00	0.91
3	0.20	6.25	10.67	4.00	1.82
4	0.30	9.09	16.00	6.00	2.73
5	0.40	11.76	21.33	8.00	3.64
6	0.57	15.97	30.40	11.40	5.18

[a] Experiments were conducted to determine if the water content (84 – 92% weight) of sludges was adequate for Bligh & Dyer extraction and to establish the number of extraction stages to effectively recover the lipidic materials in activated sludges.
[b] Blank/Control run.
[c] Water content of all samples = 3.0 mL (~3.0 grams).
[d] Single extraction. Total volume of solvent used = 18.75 mL (water content of samples not included).
[e] Double extraction. Total volume of solvent used = 50.00 mL (water content of samples not included).
[f] Five-stage extraction. Total volume of solvent used = 110.00 mL (water content of samples not included).

Methods

Single Extraction

To each of the samples, 7.5 mL of methanol and 3.75 mL of chloroform were added. Samples were then vortex-mixed for 30 minutes for homogenization. Then, 3.75 mL of water and 3.75 mL of chloroform were added and the mixtures were again vortex-mixed for 2 minutes. Phases were separated by centrifugation at 3000 rpm for 10 minutes after which, the organic (chloroform) layer was recovered. The solvent was evaporated to recover the extractable (lipidic) materials.

Double Extraction

Another set of samples (as in single extraction) was subjected to second extraction. After the removal of the organic layer, 9.50 mL of methanol and 4.75 mL of chloroform were added to the raffinate (aqueous layer). The mixture was vortex-mixed for 30 minutes and then 8.5 mL of water and 8.5 mL of chloroform were added. The mixture was homogenized and centrifuged to separate the phases. The lower extract-rich layer was combined with the first one and the solvent was removed to recover the extractable materials

Five-stage Extraction

The aqueous phases from the second extractions were subjected to three more extractions. Each extraction was conducted by addition of 20 mL chloroform to the aqueous phases followed by vortex-mixing, then by phase separation.

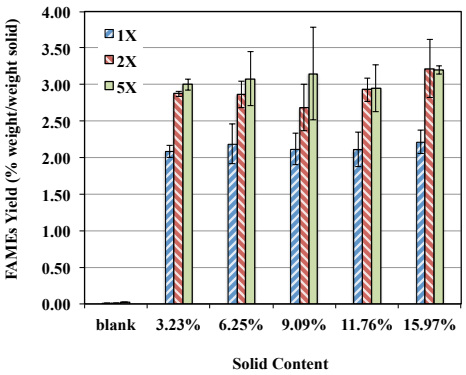
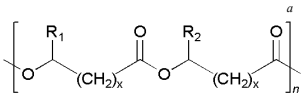
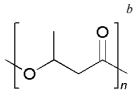
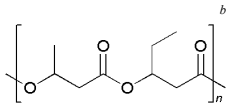
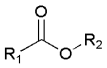
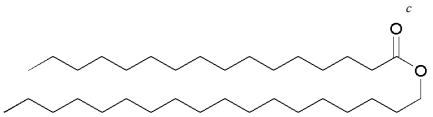
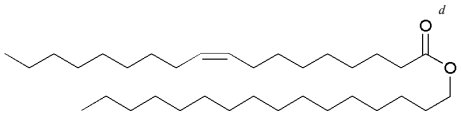
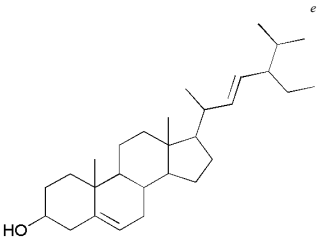
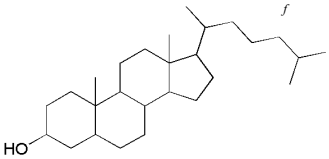
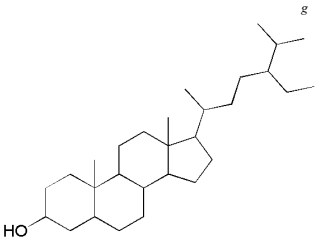


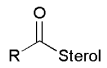
Fig. S1 FAMEs yield as a function of solid content and number of Bligh & Dyer extraction stages. Single Extraction (1X): 18.75 mL Extraction solvent. Double Extraction (2X): 50 mL Extraction solvent. Five-stage extraction (5X): 110.00 mL Extraction solvent. Water content of samples: 3.00 mL. Extraction Temperature: ambient.

Table S2 Chemical structures of different compounds present in municipal activated sludge in addition to alkanes, fatty acids, triacylglycerides, diacylglycerides, monoacyglycerides and phospholipids.

Compound Class	General Structure	Example	Structure
Polyhydroxyalkanoates	 [a] R_1/R_2 = alkyl groups from C_1 to C_{13} x = 1 - 4 n = 100 - 30000	Polyhydroxybutyrate	 [b] $R_1 = R_2 = CH_3$ x = 1
		Poly(hydroxybutyrate-*co*-hydroxyvalerate)	 [b] $R_1 = CH_3$ $R_2 = CH_2CH_3$ x = 1
Wax Esters (Waxes)	 [c] R_1/R_2 = alkyl groups	Stearyl Palmitate	 [c]
		Palmityl Oleate	 [d]
Sterols	-	Stigmasterol	 [e]
		Coprostanol	 [f]
		Stigmastanol	 [g]

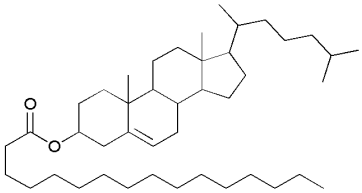
[h]

Steryl Esters



R = alkyl group

Cholesteryl Palmitate



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[d] R. Kalscheuer, T. Stöveken, H. Luftmann, U. Malkus, R. Reichelt and A. Steinbüchel, *Appl. Environ. Microbiol.*, 2006, **72**, 1373-1379.
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[f] M. M. Ehlers, A. Sundram, M. M. Kock and N. Potgieter, in *Environmental microbiology research trends*, ed. G. V. Kurladze, Nova Science Publishers, Inc., New York, 2007, ch. 4, pp. 137-166.
[g] R. J. Cole, M. A. Schweikert and B. B. Jarvis, in *Handbook of secondary fungal metabolites*, Academic Press, Amsterdam; Boston, 2003, vol. 2, ch. 3, pp. 91-126.
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Table S3 Analytical equipments and conditions.

Analyte(s)	Equipment(s)	Injector	Detector	Column	Oven
Fatty Acid Methyl Esters	Agilent 6890N GC	260°C	Flame Ionization Detector, 260°C	Restek Stabilwax-DA capillary column, 30m × 0.25mm ID, 0.25µm film thickness	Initial temperature: 50°C, 2 minutes Program 1: 250°C at 10°C/minute Final time: 18 minutes
Fractions 1 - 4	Varian 3600 GC	Cool-on-column, 50°C to 380°C, at 180°C/min.	Flame Ionization Detector, 380°C	Rtx®-Biodiesel TG, 15m × 0.32mm ID, 0.10µm film thickness with 2m × 0.53mm Rxi® guard column	Initial temperature: 50°C, 1 minute Program 1: 180°C at 15°C/minute Program 2: 230°C at 7°C/minute Program 3: 370°C at 20°C/minute Final time: 11.20 minutes
Hydrocarbons	Varian 3400 GC Agilent 6890N GC	260°C	Saturn 2000 ion-trap mass spectrometer Flame Ionization Detector, 260°C	Restek Stabilwax-DA capillary column, 30m × 0.25mm ID, 0.25µm film thickness	Initial temperature: 50°C, 2 minutes Program: 250°C at 2°C/minute Final time: 18 minutes
Sterols and Fatty Alcohols	Agilent 6890N GC	280°C	5975 inert Mass Selective Detector (mass spectrometer)	Restek Rxi®-1MS, 10m × 0.10mm ID, 0.10µm film thickness	Initial temperature: 50°C, 1.50 minutes, Program 1: 100°C at 35°C/minute Program 2: 310°C at 20°C/minute Final time: 5 minutes