J Amer Oil Chem Soc (2007) 84:181–187
DOI 10.1007/s11746-006-1022-4

ORIGINAL PAPER

Extraction of Lipids from Municipal Wastewater Plant Microorganisms for Production of Biodiesel

Stephen Dufreche · R. Hernandez · T. French · D. Sparks · M. Zappi · E. Alley

Received: 30 May 2006 / Accepted: 3 November 2006 / Published online: 3 January 2007

Abstract Municipal wastewater treatment plants in the USA produce over 6.2×10^6 t of dried sewage sludge every year. This microorganism-rich sludge is often landfilled or used as fertilizer. Recent restrictions on the use of sewage sludge, however, have resulted in increased disposal problems. Extraction of lipids from sludge yields an untapped source of cheap feedstock for biodiesel production. Solvents used for extraction in this study include *n*-hexane, methanol, acetone, and supercritical CO_2. The gravimetric yield of oil was low for nonpolar solvents, but use of polar solvents gave a considerably increased yield; however, the percentage of saponifiable material was less. Extraction of lipids with a mixture of *n*-hexane, methanol, and acetone gave the largest conversion to biodiesel compared with other solvent systems, 4.41% based on total dry weight of sludge. In situ transesterification of dried sludge resulted in a yield of 6.23%. If a 10% dry weight yield of fatty acid methyl esters is assumed, the amount of biodiesel available for production in the USA is 1.4×10^6 m^3/year. Outfitting 50% of municipal wastewater plants for lipid extraction and transesterification could result in enough biodiesel production to replace 0.5% of the national petroleum diesel demand (0.7×10^6 m^3).

Keywords Bacterial lipid extraction · Biodiesel · Biosolids · In situ transesterification · Municipal sewage sludge · Solvent extraction

S. Dufreche · R. Hernandez (✉) · T. French · D. Sparks · E. Alley
Renewable Fuels and Chemicals Laboratory, Chemical Engineering Department, Mississippi State University, Mail Stop 9595, Mississippi State, MS 39762, USA
e-mail: rhernandez@che.msstate.edu

M. Zappi
University of Louisiana, College of Engineering, P.O.Box 42251, Lafayette, LA 70504, USA

Introduction

Activated sludge is the solid or semisolid produced during biological treatment of industrial and municipal wastewaters. It contains a variety of microorganisms, which utilize the organic and inorganic compounds in the water as a source of energy, carbon, and nutrients. The waste sludge containing 1–2% solids is usually concentrated via gravity-thickening or air-floatation to approximately 10% solids. In many cases, the concentrated sludge is introduced into an aerobic or anaerobic digester to reduce the level of pathogens and odors (stabilization). In a wastewater treatment facility, activities associated with sludge treatment represent from 30 to 80% of the electrical power consumed at the plant [1].

Wastewater treatment facilities in the USA produce approximately 6.2×10^6 t (dry basis) of sludge annually [1]. Prior to or after stabilization, the sludge may be dewatered and disposed of via incineration, land application, or placement in landfills; however, several environmental health and safety concerns restrict the feasibility of these options.

One new method of disposal is production of bio-oil via thermal conversion or pyrolysis [2]; the resulting liquid has a high-heating value and potential

for electricity generation. However, this process has only been moderately successful since the complexity of bio-oil complicates the chemical processes needed to refine it.

Research has recently indicated that the lipids contained in sewage sludge are a potential feedstock for biodiesel. Biodiesel, a mixture of fatty acid alkyl esters, is produced from vegetable oils or animal fats [3]. The literature indicates that sewage sludge contains approximately 20% ether-soluble grease and fats [4], which could be converted into fatty acid methyl esters (FAMEs). Production of biodiesel is via base- and/or acid-catalyzed transesterification of the triglycerides, diglycerides, monoglycerides, phospholipids, and free fatty acids contained in oils and fats. Additionally, the cell membrane of microorganisms, the main component of sewage sludge, is mostly composed of phospholipids [5], which can be converted to fatty acid alkyl esters via acid- and base-catalyzed transesterification. Glycerol is a by-product generated in both reactions and is used by industry for manufacturing cosmetics and pharmaceutical formulations. If it is assumed that cells contain 2% phosphorous by dry weight with 50% of phosphorous in the cell membrane, calculations on the estimated mass of phospholipids in cells place their content at 24% by dry mass. This is in agreement with literature values of 25% for *Escherichia coli* [6].

The establishment and growth of the biodiesel industry has increased demand for raw materials over the last 5 years. Most biodiesel in the USA is produced using soybean oil. In 2004, the total US soybean production was 85.49×10^6 t, while domestic consumption was 44.91×10^6 t. From the surplus of 10.64×10^6 t, approximately 2.66×10^6 t of oil (2.9×10^6 m^3) was available for biodiesel production [7]. This would yield approximately 2.64×10^6 m^3 of biodiesel and 0.264×10^6 m^3 of glycerin. In 2004, the total petroleum diesel consumption in the USA was approximately 150×10^6 m^3. Only 1.8% petroleum displacement could be achieved by transforming all the non-food-related soybean oil into biodiesel. New oil sources will have to become available for biodiesel to displace significant amounts (more than 5%) of petroleum from the market.

The main objective of this paper is to demonstrate that sewage sludge can be a raw material for the production of FAMEs. Lipids were extracted using organic solvents with different polarities or supercritical carbon dioxide (SC-CO_2). The paper provides results on the lipid and FAME yields obtained with several extraction strategies.

Experimental Procedures

Chemicals

Methanol, acetone, 1,3-dichlorobenzene, sulfuric acid, sodium chloride, and *n*-hexane were purchased from Fisher Scientific, Atlanta, GA, USA. Industrial-grade carbon dioxide was provided by NexAir, Memphis, TN, USA. These chemicals were used as received.

Sewage Sludge

Secondary sewage sludge was collected from a municipal wastewater treatment plant located in Tuscaloosa, AL, USA. It was collected from the aerobic waste sludge line, which fed into the anaerobic digester.

Sample Preparation

Upon collection at the facility, the sludge flocks were allowed to undergo gravity-settling. Separation of the clarified water resulted in a sludge containing 2% solids. This sludge was dewatered by centrifugation or pressure filtration. Centrifugation was performed with a Marathon 3000 centrifuge manufactured by Fisher Scientific and operated at 3,000 rpm for 20 min. Removal of the free water resulting from this step gave a sludge containing 7–8% solids. Pressure filtration was conducted with a Millipore 1.5-L pressure filter pressurized in 69-kPa increments from 103 to 517 kPa. Sludge was first filtered using an 80-μm nylon filter, with the filter cake collected for later use. The filtrate was then filtered again with a 20-μm nylon filter and the cake was combined with that from the 80-μm run. The remaining sludge cake contained 12–14% solids.

Organic-Solvent Extraction

Prior to organic-solvent extraction, dewatered sludge was mixed with Hydromatrix (manufactured by Varian, Palo Alto, CA, USA) and loaded into a steel sample vessel. The Hydromatrix absorbed residual free water in the sample and competed for bound water during extraction. Hydromatrix was added until the sample formed small pellets and flowed freely. Solvent extraction was conducted using a 200 series accelerated solvent extraction system (manufactured by Dionex, Sunnyvale, CA, USA), which included a multisolvent control system. The system was operated at 10.3 MPa and 100 °C for 1 h per extraction. Single or sequential (two or three times) extractions were examined using

the following solvent mixtures (percentage by volume) or pure solvents:

1. 60% hexane/20% methanol/20% acetone (HMA) (same mixture three times)
2. Pure methanol followed by pure hexane (MH)
3. Pure hexane (single extraction)
4. Pure methanol (single extraction)

After extraction, the sample vessel was drained into a glass collection vial, followed by a solvent flush equal to 50% of the sample vessel's volume. The lipid-containing solvent vial was then stored at –15 °C until further analysis. Each experiment was performed in duplicate, except for extraction with pure hexane, which was a singlet.

SC-CO_2 Extraction

A fluid-bed dryer (model FBD 2000, Endecotts, London, UK) and an oven (Fisher Scientific, Atlanta, GA, USA) were utilized to reduce the moisture content of the sludge to approximately 5% moisture prior to extraction using SC-CO_2. The moisture content was determined using IR heating (model MB45, Ohaus, Pine Brook, NJ, USA). The SC-CO_2 extractions were performed with a laboratory-scale extractor manufactured by Thar Technologies (model SFE 100; Pittsburgh, PA, USA). The extractions were conducted at 45 °C and 32.5 MPa. Four sequential extractions were performed on each sludge sample. In the case where methanol was used as a cosolvent, the flow rate was based on the total weight of the solvents, 3.75 and 0.50 g/min. The oil-collection vessel was rinsed with 20 mL hexane when using purely SC-CO_2 as the extraction medium. Methanol was used in addition to hexane to rinse the vessel after the cosolvent experiments to dissolve any polar lipids. Owing to constraints on the amount of sludge required for sample analysis, the extractions were only performed once per condition.

In Situ Transesterification

A fluid-bed dryer was used in the same manner as in SC-CO_2 extraction to achieve a sample with 5% moisture content. The dried sludge was then ground in a mortar and pestle until a fine powder was obtained. A screw-top vial was then charged with 1 mL of 1% sulfuric acid in methanol and 200 mg of powdered sample. The vial was then capped and heated overnight at 50 °C. Then, a 5-mL aliquot of 5% NaCl in water was added and the FAMEs were extracted with hexane (2×5 mL), vortexing the vial between extractions to provide efficient mixing. The hexane phase was washed with 2% sodium bicarbonate and dried over sodium sulfate. The experiment was performed in duplicate.

Sample Analysis

After extraction, the lipid-containing solvent phase was removed under vacuum in a Büchi R205 rotary evaporator (rotovap) at 40 °C and 15–30 kPa of vacuum. The resulting lipid was weighed using an Ohaus analytical balance. The yield of extracted material was then determined and expressed as grams of extractable lipid per gram of dry solid.

Conversion of the lipids to FAMEs for extraction with organic solvents was carried out through acid catalysis using a modified version of Christie's method [8]. Twenty milligrams of lipids was dissolved in 1 mL of hexane containing 1,3-dichlorobenzene as an internal standard and added to a vial with 2 mL of 1% sulfuric acid in methanol. The vial was then capped and heated overnight at 50 °C. Then, a 5-mL aliquot of 5% NaCl in water was added and the FAMEs were extracted with hexane (2×5 mL), vortexing the vial between extractions to provide efficient mixing. The hexane phase was washed with 2% sodium bicarbonate and dried over sodium sulfate.

Transesterification of lipids from SC-CO_2 extractions was performed in an Erlenmeyer flask. The flask was charged with 0.1–0.2 g of lipid and 10.0 mL of *n*-hexane. Four milliliters of 0.5 N sodium methoxide was then added and the mixture was refluxed for 10 min. The flask was allowed to cool and 5.0 mL of 14.0% BCl_3 was added. The mixture was refluxed for 10 min and was dried by filtering through sodium sulfate.

The FAMEs produced by transesterification were analyzed using an Agilent gas chromatograph (model 6890; Palo Alto, CA, USA) with a flame-ionization detector. Helium was used as the carrier gas. The separation was achieved with a fused-silica capillary column composed of stabilized 90% polybiscyanopropyl/10% cyanopropylphenyl siloxane (SP-2380; Supelco, Bellefonte, PA, USA). The dimensions of the column were 100 m × 0.25 mm, with a phase thickness of 0.2 μm. A calibration curve was prepared by injecting known concentrations of an external standard mixture comprising 37 FAMEs (47885-U, 37 Component FAME Mix; Supelco, Bellefonte, PA, USA). All calibration curves were linear with a correlation coefficient of 0.99 or better. 1,3-Dichlorobenzene was used as an internal standard. The method consisted of injecting 1 μL of sample into the gas chromatograph with a split ratio of 100:1. The temperature program began at 110 °C, holding for 2 min. It then increased by

10 °C/min to 140 °C, where it was held for 4 min. After 9 min of total run time, the temperature increased 2 °C/min until reaching 240 °C. The temperature was then held constant until a total run time of 99 min was achieved. Concentration data obtained from gas chromatography runs were used to calculate the amount of saponifiable material in extracted lipids. Only compounds with a concentration greater than 1% were counted toward the total FAME.

Results and Discussion

The amounts of oil, saponifiable material, and the overall yield obtained through different extraction methods can be found in Table 1. Different solvents were used during extraction to determine which system gave the best yield. An explanation of the difference in extraction yield by solvent can be rationalized through use of the Hildebrand solubility parameter (δ), which is a measure of the ''strength'' of the solvent [9]. This can be thought of as the energy required to create a ''hole'' in the solvent for another molecule to fit in. It can be broken into three parts called the Hansen parameters, which describe forces acting on a molecule. The dispersion force is a measure of London dispersion forces, or nonpolar interaction, given by δ_d. The magnitude of the dipole moment contribution is given by δ_p, and the hydrogen-bonding contribution is represented as δ_h. The summed squares of these parameters are equal to the square of the total Hildebrand solubility parameter, $\delta^2 = \delta_d{}^2 + \delta_p{}^2 + \delta_h{}^2$. Solvents with similar Hildebrand parameters are usually miscible with each other, although the individual Hansen parameters must also be taken into account. The behavior of solutes can also be predicted in the same way [9]. Table 2 gives Hansen parameters for the solvents used in extraction of lipids from sludge.

As Table 2 shows, all of the solvents have roughly equal contributions from dispersion forces, with the exception of SC-CO_2. It is also evident that acetone and methanol are almost equal in strength regarding polarity, while *n*-hexane has no polar force at all. The degree of hydrogen bonding is greatest for methanol and is less for acetone. The difference in solvents can thus be summed up as follows. *n*-Hexane contains only dispersion forces and may be considered a standard solvent used for comparison. Acetone and methanol are used to examine the effect of highly polar solvents on extraction and low and high hydrogen-bonding strengths. SC-CO_2 has the smallest dispersion value, with a polarity around half that of acetone and methanol and a hydrogen-bonding strength near that of acetone.

Table 1 shows that when used in a single solvent extraction, hexane achieves an oil yield of 1.94%, while SC-CO_2 gives 3.55%. However, the addition of a polar cosolvent in both the conventional and the SC-CO_2

Table 1 Extraction and transesterification yield of waste activated sludge

Extraction medium	Oil yield[a] (%)		Oil saponifiable[b] (%)		Overall yield[c] (%)	
100% hexane[d]	1.94		19.7		0.38	
100% methanol[d]	19.39 ± 3.20		14.25 ± 1.66		2.76 ± 0.39	
Extraction 1—HMA[e]	21.20	27.43 ± 0.98	16.22	16.18 ± 3.21	3.44	4.41 ± 0.63
Extraction 2— HMA	5.37		15.57		0.84	
Extraction 3— HMA	0.86		15.92		0.14	
Extraction 1—100% methanol[f]	19.39	21.96 ± 2.28	14.25	14.21 ± 1.53	2.76	3.07 ± 0.33
Extraction 2—100% hexane	2.57		12.03		0.31	
SC-CO_2	3.55		7.87		0.28	
SC-CO_2 w/1.96 wt% MeOH	4.19		26.8		1.12	
SC-CO_2 w/13.04 wt% MeOH	13.56		17.0		2.31	
In situ transesterification[g]	–		–		6.23 ± 0.11	

All extractions were carried out at 100°C for 1 h; solvent-to-solids ratio 40:1 g/g

[a] Gravimetric yield of oil in grams of oil per gram of dry sludge. Values on the *left* indicate individual extraction yields; values on the *right* indicate total yield

[b] Percentage of extracted oil saponifiable on a mass basis. Values on the *left* indicate individual extraction yields; values on the *right* indicate total yield

[c] Grams of fatty acid methyl ester produced per 100 g of dry sludge

[d] Sample extracted once

[e] Sample extracted three times; *HMA* 60% hexane/20% methanol/20% acetone, *SC-CO₂* supercritical CO_2

[f] Sequential extraction using methanol followed by hexane

[g] Dried to 95 wt% solids. The solvent was methanol with 1% sulfuric acid

Table 2 Solubility parameters for solvents and solvent systems employed in extraction

Solvent	δ_d[a] ($MPa^{1/2}$)	δ_p[b] ($MPa^{1/2}$)	δ_h[c] ($MPa^{1/2}$)	δ[d] ($MPa^{1/2}$)
Acetone[e]	15.5	10.4	7.0	20.0
Methanol[e]	15.1	12.3	22.3	29.6
n-Hexane[e]	14.9	0.0	0.0	14.9
SC-CO_2[f]	11.9	4.7	5.1	13.7
HMA[e]	15.1	7.1	10.6	19.8

[a] Magnitude of London dispersion forces

[b] Magnitude of dipole moment contribution

[c] Magnitude of hydrogen-bonding contribution

[d] Combined solubility parameter

[e] Calculated using values from Hansen [9]

[f] Calculated using values from Croudace and Ritz [10]

system resulted in an increase of extracted oil. Compared with extraction with pure hexane, a single extraction using a mixture of hexane, methanol, and acetone increased the oil yield from 1.94 to 21.20%. Following the same trend, adding increasing amounts of methanol to SC-CO_2 (0, 1.96, and 13.04 wt%) increased the yield of oil from 3.55 to 4.19 and 13.56%. In addition, the sequential extraction experiment of a hexane, methanol, and acetone mix shows that a significant amount of material is left behind after the first extraction. However, the amount of extractable oil decreases sharply with each subsequent extraction. The increase in yield due to addition of polar cosolvents could be due to high phospholipid levels in the sample. Phospholipids have a polar head and a nonpolar tail. Secondary sludge is mainly composed of microorganisms whose cell membranes contain phospholipids. Addition of the methanol/acetone mix would expose phospholipids to a solvent with high Hansen values for polarity and hydrogen bonding. It is hypothesized that the solvent mixture helps to disrupt the lipid membrane, which is held together through hydrophobic interactions and is protected by polar head groups. Samples of extracted lipid analyzed through thin-layer chromatography indicated the presence of phospholipids, but quantitative amounts could not be obtained.

If one extracts with a pure polar solvent instead of a nonpolar one, the oil yield is much larger. An extraction with pure methanol gives 19.39% yield compared with the 1.94% yield with pure hexane. This reinforces the idea of polar lipids being extracted more easily with a polar solvent. Following the pure methanol extraction with pure hexane gives a yield for hexane of 2.57%, which is slightly higher than the yield obtained for extraction with hexane on a virgin sample. This is intuitive if one considers that hexane extracts mainly nonpolar lipids with low values of δ_p and δ_h, while methanol prefers polar lipids with larger values of δ_p and δ_h. Extraction with a polar solvent first may help destroy the cellular membrane and allow a subsequent nonpolar extraction access to previously unreachable lipids within the cell.

While polar solvents show a large increase in extractable oil yield, the percentage of saponifiable material is lower. Conversion of a pure hexane extract to FAMEs gives 19.70% saponification by weight of the material extracted, while conversion of a pure methanol extract only gives 14.25%. Extracting with a mixture of solvents such as the HMA system results in a transesterification yield of 16.22% of material extracted, lower than with pure hexane but higher than with pure methanol. Repeated extraction with the HMA system shows that the percentage of saponifiable material in the extracted oil does not change much with subsequent extractions. The percentage of saponifiable material extracted in the MH system is greater for methanol than for hexane; however, the percentage of saponifiable material for hexane on a sample already extracted with methanol is lower than for a virgin hexane extraction. This suggests that treatment with a polar solvent will help disrupt cell walls, releasing more extractable material than just nonpolar lipids. Analysis of the SC-CO_2 extractions shows an increase in saponifiable material with the addition of a small amount of methanol. Continuing to increase the methanol content will result in a larger oil yield but a lower percentage of transesterifiable material. The trend of decreasing transesterification yield with increasing amounts of polar solvent can also be rationalized through use of the Hildebrand solubility parameters. Hexane has no Hansen components for polarity or hydrogen bonding, and a low total Hildebrand value. This gives hexane the ability to extract compounds with similar Hildebrand parameters, including nonpolar lipids such as triglycerides. In contrast, methanol is highly polar and has a high degree of hydrogen bonding. This allows for extraction of polar groups such as those found on phospholipids and nonlipid compounds found throughout the bacterial cell. The more polar solvents extract larger amounts of nonlipid material, causing a sharp increase in oil yield, which is measured on a weight basis. This is accompanied by a decrease in the percentage of saponifiable material.

Calculations based on the amount of oil extracted and the percentage of saponifiable material in the oil give an overall yield of saponifiable material extracted from the sludge. This is represented as the mass of biodiesel produced per mass of dry sludge. Although the percentage of saponifiable material in a pure

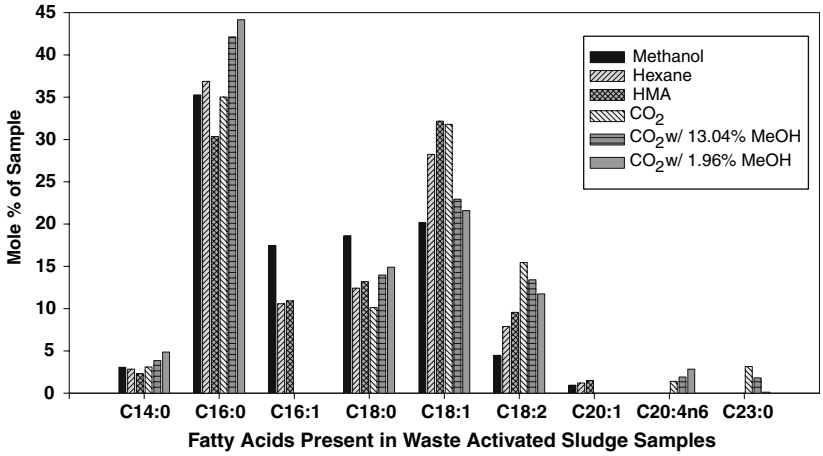

Fig. 1 Impact of extraction medium on fatty acid composition of oil

hexane extraction was higher than that in a pure methanol extraction, the methanol gives a higher overall yield owing to a much larger amount of oil extracted. This shows that while an extraction with a polar solvent will produce oil heavily contaminated with nonsaponifiable material, the total amount of saponifiable material is larger. The increase can be attributed to greater extraction of phospholipids with methanol than with hexane. Since phospholipids contain a maximum of two fatty acid groups per molecule, the yield of biodiesel is reduced. A comparison of the HMA extraction with the MH extraction shows that the first HMA extraction gives a slightly higher overall yield than the MH extraction. Combining the last two HMA extractions gives the system a significantly larger yield than the MH extraction. The overall yield from the SC-CO_2 extraction is the lowest of all the extractions, but increases as methanol is added. At 13.04 wt% methanol, the overall yield increased to 2.31%.

The last row in Table 1 refers to in situ transesterification of dried sludge. This is a method in which the lipids are converted to FAMEs without extracting them from the sludge. Since the reagents have access to all oil in the sludge instead of just what was extracted, the yield should be higher than with the other methods. Indeed, the yield of 6.23% is the highest of all methods tried.

An analysis of the fatty acid profile in Fig. 1 shows differences in lipid composition as a function of various extraction methods. Examination of the major fatty acid components shows that the SC-CO_2 extraction gives lower levels of unsaturated fatty acids when methanol is added to the system. The profile for hexane and methanol also shows that hexane extracts a larger ratio of unsaturated fatty acids than methanol for all compounds except the C16s. In addition, all SC-CO_2 extractions appear to favor polyunsaturated fatty acids more than conventional extraction with hexane and methanol. This can be seen in Fig. 2, where SC-CO_2 extraction averages 16% polyunsaturated fatty acids against 10% for hexane and methanol. If the profile of in situ is taken to be the baseline, the SC-CO_2

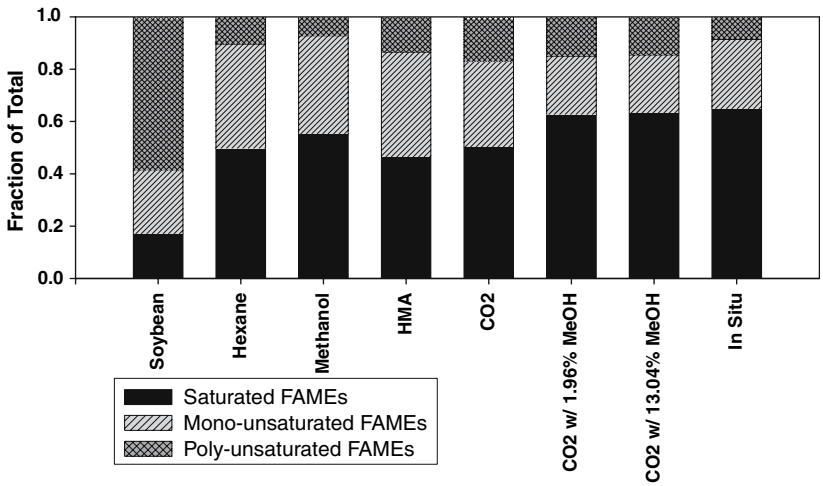

Fig. 2 Comparison of saturated versus unsaturated fatty acids present in waste activated sludge samples

Table 3 Production cost estimate for sludge biodiesel

	Cost per gallon ($)
Centrifuge O&M	0.43
Drying O&M	1.29
Extraction O&M	0.34
Biodiesel processing O&M	0.60
Labor	0.10
Insurance	0.03
Tax	0.02
Depreciation	0.12
Capital P&I service	0.18
Total cost	3.11

Assuming 7.0% overall transesterification yield

O&M operation and maintenance, *P&I* protection and indemnity

systems extract higher amounts of polyunsaturated fatty acids from the sludge than conventional solvents, while leaving some saturated fatty acids behind in the sample. Comparison of sludge fatty acid profiles by various extraction methods with the profile of a standard soybean sample shows that all sewage sludge samples have a much larger concentration of saturated fatty acids. Although the higher levels of saturated fatty acids may present a problem in cold weather owing to gelling, the higher saturated content will produce a better burning biodiesel [10].

Examination of the various transesterification methods shows that in situ conversion of lipids to FAMEs provides the highest overall yield of biodiesel. A breakdown of processing cost is shown in Table 3. If we assume a 7.0% overall yield of FAMEs from dry sewage sludge on a weight basis, the cost per gallon of biodiesel would be $3.11. As transesterification efficiency increases, the cost per gallon drops quickly, hitting $2.01 at 15.0% overall yield. An overall yield of 10.0% is required to obtain biodiesel at $2.50 per gallon, allowing it to compete with soy biodiesel in the marketplace. The authors feel that with further optimization such a yield is easily obtainable.

Acknowledgements This work was supported by research grants from the Department of Energy (DE-FG36-04GO14251) and DOE EPSCOR (DE-FG02-00ER45830). Jessy Avelar and Keith Barlow provided laboratory assistance.

References

1. Water Environment Federation (2002) Activated sludge MOP OM-9, 2nd edn. Water Environment Federation
2. Konar SK, Boocock D, Maom V, Liu J (1994) Fuels and chemicals from sewage sludge: 3. hydrocarbon liquids from the catalytic pyrolysis of sewage sludge lipids over activated alumina. Fuel 73:642–646
3. Ma F, Hanna M (1999) Biodiesel production: a review. Bioresour Technol 70:1–15
4. Loehr R, Navarra C Jr (1959) Grease removal at a municipal treatment facility. J WPCF 41:142–154
5. Rittmann B, McCarty P (2001) Environmental biotechnology: principles and applications. McGraw-Hill, New York
6. Nelson D, Cox M (2005) Principles of biochemistry. Freeman, San Francisco
7. The American Soybean Association (2005) Soy stats 2005. http://www.soystats.com/2005/Default-frames.htm
8. Christie W (2003) Lipid analysis. The Oily Press, Bridgwater
9. Hansen CM (2000) Hansen solubility parameters: a user's handbook. CRC, Boca Raton
10. Croudace M, Ritz G (2003) Biodiesel/FAME analysis. Hydrocarb Eng 8:83–84