Energy Recovery from Municipal Sewage Sludge: An Environmentally Friendly Source for the Production of Biochemicals

Luigi di Bitonto*, **Antonella Angelini** and **Carlo Pastore**

Water Research Institute (IRSA), National Research Council (CNR), Viale De Blasio 5, 70132 Bari, Italy
Corresponding author: luigi.dibitonto@ba.irsa.cnr.it

S.1 Materials and Methods

S.1.1 Reagents and Instruments

Methanol (99%), hexane (99%), (S)-1-phenyl-propyl-isocyanate (>96%), (R)-1-phenyl-methyl-isocyanate (>95%) and methyl heptadecanoate (≥99%) were purchased as pure-grade reagents (Sigma-Aldrich) and used directly without further treatment. Aluminium chloride hexahydrate (99%) was purchased from Baker. Formic acid (99%), potassium hydroxide (85%), diethyl ether (99%), dichloromethane (99%), acetic acid (99.5%), nitric acid (65%), hydrochloric acid (HCl, 37%), phenolphthalein and methyl-red were obtained as pure-grade reagents (Carlo Erba). For silylation, N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA, ≥99%) and pyridine were purchased from Sigma-Aldrich. (S)-(+)-α-methoxyphenylacetic acid, dicyclohexylcarbodiimide and 4-(N,N-dimethylamino)-pyridine were purchased from Alfa Aesar. A Rotofix 32 Hettich Centrifuge was used for the centrifugation experiments. Gas chromatography-mass spectroscopy (GC-MS) for qualitative analysis was carried out using a Perkin Elmer Clarus 500 gas-chromatograph connected to a Clarus 500 spectrometer. Quantitative determinations were performed with a Varian 3800 GC-FID. The instrument was configured for cold on-column injections with an HP-5MS capillary column (30 m; 0.32 mm, 0.25 μm film). Both the injector and the oven followed the same temperature program: an initial temperature of 60 °C was kept constant for 2 min and then increased with a ramp of 15 °C min^{-1} until the final temperature of 300 °C was reached. A Helium (He) flow of 1.3 mL min^{-1} was used as a carrier. For GC-MS, the ion source was operated at 70 and 45 eV and maintained at 250 °C. Gas-chromatographic analysis of the diastereomers was performed on a HP-5MS capillary column (60 m; 0.25 mm, 0.25 μm film). The initial temperature was set to 90 °C and kept constant for 2 min. Subsequently, the temperature was first increased to 200 °C (with a ramp of 15 °C min^{-1}) and then to 280 °C afterwards (through a 0.1 °C min^{-1} ramp). Infrared spectra (FTIR) of isolated products were recorded using a Nicolet iS10 Thermo Scientific spectrometer with a resolution of 4 cm^{-1} equipped with a DTGS KBr detector.

S.1.2 Total lipids

20.0 g of wet primary sludge (or primary sludge) were placed in a tube of 50 mL containing 20 mL of hexane and shaken at room temperature for 2 h. The upper organic suspension was collected and set aside. This procedure was repeated four times and all the organic fractions were collected in a single glass vessel. The hexane was evaporated under nitrogen flow, and the final residue was quantified gravimetrically so that the corresponding weight fraction, based on the dry mass, could be calculated. Finally, Free Fatty Acids (FFAs) and soaps contents, FFAs profile and the Average Molecular Weight of FFAs (AMW) were determined as described below.

S.1.3 Chemical characterization of lipids

S.1.3.1 Determination of FFAs and soaps

FFAs were determined by titration with a 0.1 N KOH-normalized solution in a 1:1 v/v diethyl ether:ethanol medium, using phenolphthalein as an indicator. 1 g of the sample was dissolved in 150 mL of the solvent. Soaps were determined under the same experimental conditions by titration with a 0.1 N HCl-normalized solution and methyl red as indicator.

S.1.3.2 Fatty Acid profile and Average Molecular Weight (AMW) determination

20 mg of the sample was placed in a 5 mL glass reactor containing 2 mL of the solution of toluene, methanol and concentrated H_2SO_4 (2:2:0.01 v/v/v). The reactor was sealed and placed in an ultrasonic bath at 70 °C for 5 h. 1 mL of a 1000 ppm methyl heptadecanoate-toluene solution was then added as an internal standard. AMW was determined by gas chromatography (1 µL) according to Eq. S1:

$$AMW = \frac{\sum A_i \, MW_i}{\sum A_i} \tag{S1}$$

where A_i and MW_i are the experimentally determined areas and molecular weights of the FFAs, respectively. Finally, total FAMEs content was calculated with respect to methyl heptadecanoate by using Eq. S2:

$$FAMEs \ content = \frac{\sum A_i}{m_{sample} \, A_{std}} \ x \ 100 \tag{S2}$$

where A_i are the area of methyl esters, A_{std} is the area referred to the standard (methyl heptadecanoate), and m_{sample} is the exact value of the sample used in the analysis.

S.1.3.3. GC-FID and GC-MS of salinized compounds

About 20 mg of sample were placed in a vial together with 100 µL pyridine and 50 µL MSTFA and were left for 1 h at room temperature. Then, 10 µL methyl heptadecanoate standard solution in undecane (1000 ppm) was added and hexane was finally added up to a final volume of 1 mL. This solution was analyzed by gas chromatography (GC-FID and GC-MS).

S.2 Characterization of reaction products obtained from esterification process
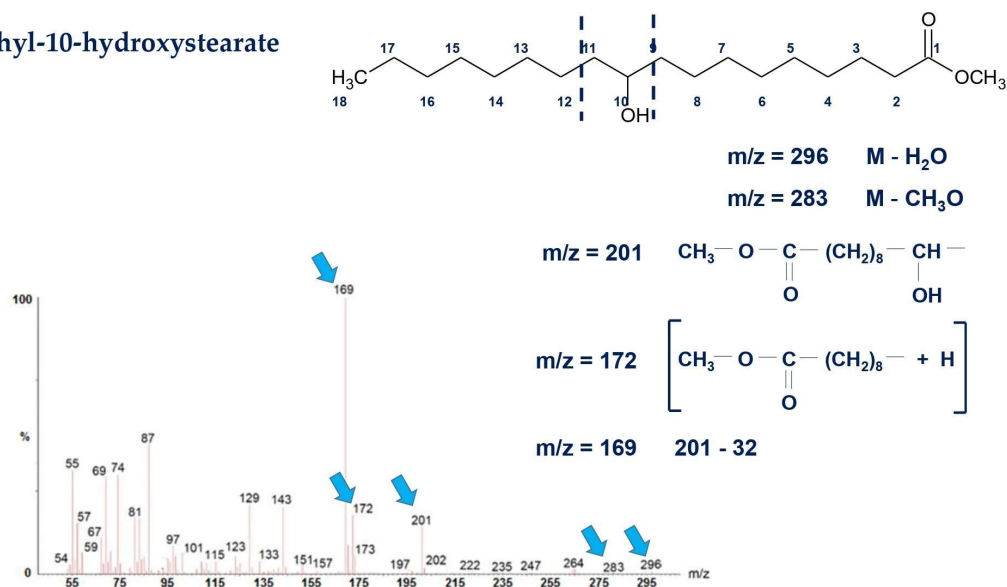
Methyl-10-hydroxystearate

$m/z = 296$ $M - H_2O$

$m/z = 283$ $M - CH_3O$

$m/z = 201$ $CH_3 - O - C - (CH_2)_8 - CH -$
                $\parallel$ O OH

$m/z = 172$ $[CH_3 - O - C - (CH_2)_8 - + H]$
                   $\parallel$
                   O

$m/z = 169$ $201 - 32$

Figure S2.1. GC-MS spectra of methyl-10-hydroxystearate.

Methyl-10-hydroxystearate
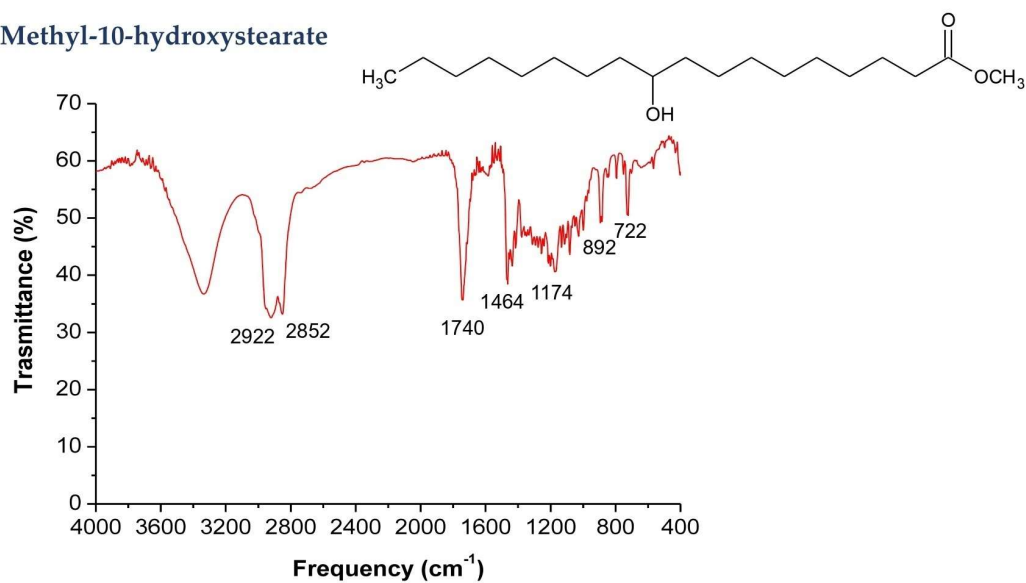
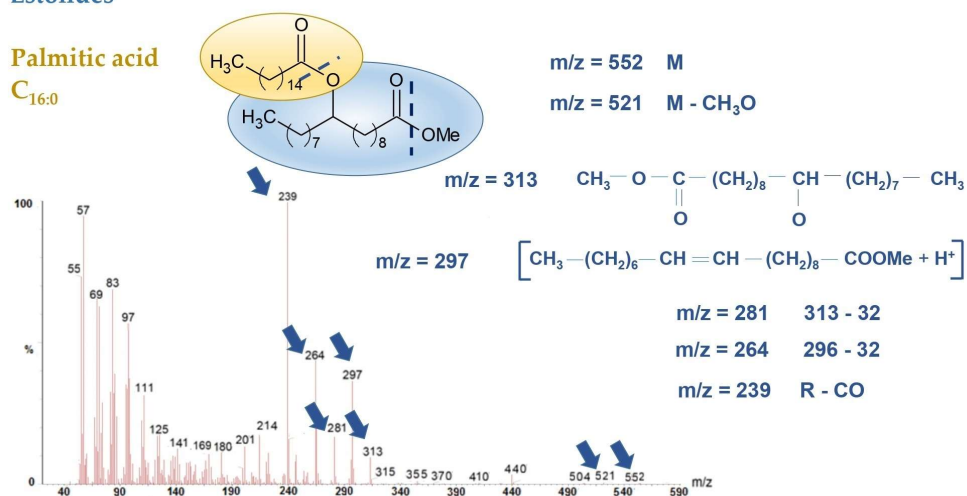**Figure S2.2.** FTIR spectra of methyl-10-hydroxystearate isolated.

Figure S2.3. GC-MS spectra of 10-(palmitoyloxy) stearic acid methyl ester.

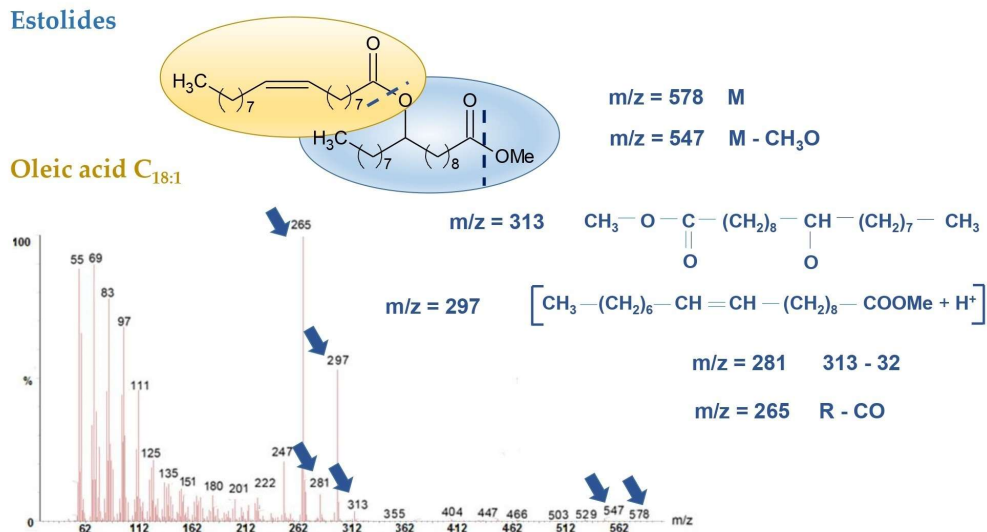**Figure S2.4.** GC-MS spectra of 10-(oleyloyloxy) stearic acid methyl ester.
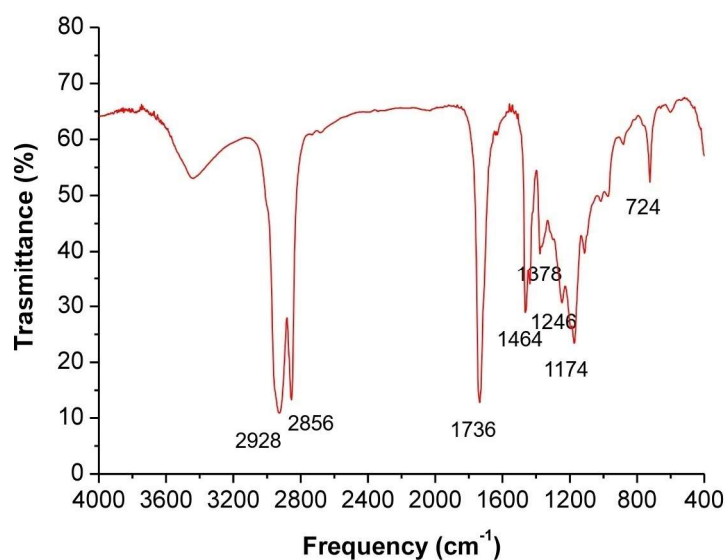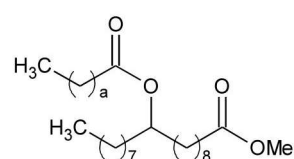
Estolides

Figure S2.5. FTIR spectra of estolides isolated.

S.3 Estimation costs of biodiesel production from primary sludge and sewage scum

Table S3.1. Summary of reagents, products and thermodynamic variables used for the determination of total energy demand of both processes.*

E	Variables	Value
Reagents		
1	Hexane	0.45 € kg⁻¹ [1]
2	Sulfuric acid	0.04 € kg⁻¹ [2]
3	Formic acid	0.8 € kg⁻¹ [3]
4	Methanol	0.36 € kg⁻¹ [4]
5	Aluminium chloride hexahydrate	0.54 € kg⁻¹ [5]
Products		
6	FAMEs	0.80 € kg⁻¹ [2]
7	Me-10-HSA	2.96 € kg⁻¹ [6]
8	Methyl estolides	2.96 € kg⁻¹ [6]
Thermodynamic variables		
9	cH_2O	4.18 kJ K⁻¹ kg⁻¹
10	cMeOH	2.47 kJ K⁻¹ kg⁻¹
11	cHexane	2.26 kJ K⁻¹ kg⁻¹
12	cFAMEs	2.248 kJ K⁻¹ kg⁻¹
13	ΔHH_2O	2257 kJ kg⁻¹
14	ΔHMeOH	1100 kJ kg⁻¹
15	ΔHHexane	365 kJ kg⁻¹
16	Aluminium chloride hexahydrate [5]	351.85 kJ kg⁻¹

* The Energetic demand per each operation was determined by the sum of Sensible heat (QSens. i= mi·ci·Δt) and Latent heat of evaporation (when necessary, QLat.i = mi·ΔHi).

References

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S.4 Analysis of biodiesel isolated by distillation process

Table S4.1. Comparison of the chemical properties of biodiesel isolate by distillation and EN14214 standard.

Properties	Biodiesel	EN14214	
		Lower limit	Upper limit
Ester content (%wt)	98.5-99	96.5	-
Water content (mg kg^{-1})	70-100	-	500
Acid value (mg KOH g^{-1})	0.2-0.3	-	0.5
Methanol content (%wt)	-	-	0.2
Monoglycerides content (%wt)	-	-	0.7
Diglycerides content (%wt)	-	-	0.2
Triglycerides content (%wt)	-	-	0.2
Free Glycerine (%wt)	-	-	0.02
Total Glycerine (%wt)	-	-	0.25
Group I metals (Na + K)	-	-	5
Group II metals (Ca + Mg)	-	-	5