Technical report on processing the activated sludge obtained from the operation of demo scale selector for lipids (UL) installed at the SIVOM de l'Alzette wastewater treatment plant

Concentration and drying of the sludge, lipids extraction, recovery and production of demo-biodiesel

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Table of contents

1 Introduction

Sewage contains valuable substances that can be used as raw materials for biobased products. However, to date this potential has hardly been exploited to its full potential in North-West Europe. This results in loss of valuable materials, CO_2-emmissions and less efficient use of natural resources. The Interreg North-West Europe project WOW! - Wider business Opportunities for raw materials from Waste water (sewage) - aims to develop three value chains for the recovery of carbon based elements from sewage (see Figure 1):

1. **The production of biodiesel**. The sewage inflow is used to cultivate Microthrix p. which can accumulate lipids. The lipids are extracted, processed and transformed to biodiesel.
2. **The production of bio-oil, biochar and acetic acid**. The screening material which mainly consists of cellulose material (toilet paper) is dewatered and dried. In a thermal degradation process (pyrolysis) the dried cellulose material is converted into biochar, bio-oil and acetic acid.
3. **The production of PHA (bioplastic)**. For this the primary sludge is used. In a biological process, PHA is enriched and extracted. Then the PHA is compounded and processed to an end product.

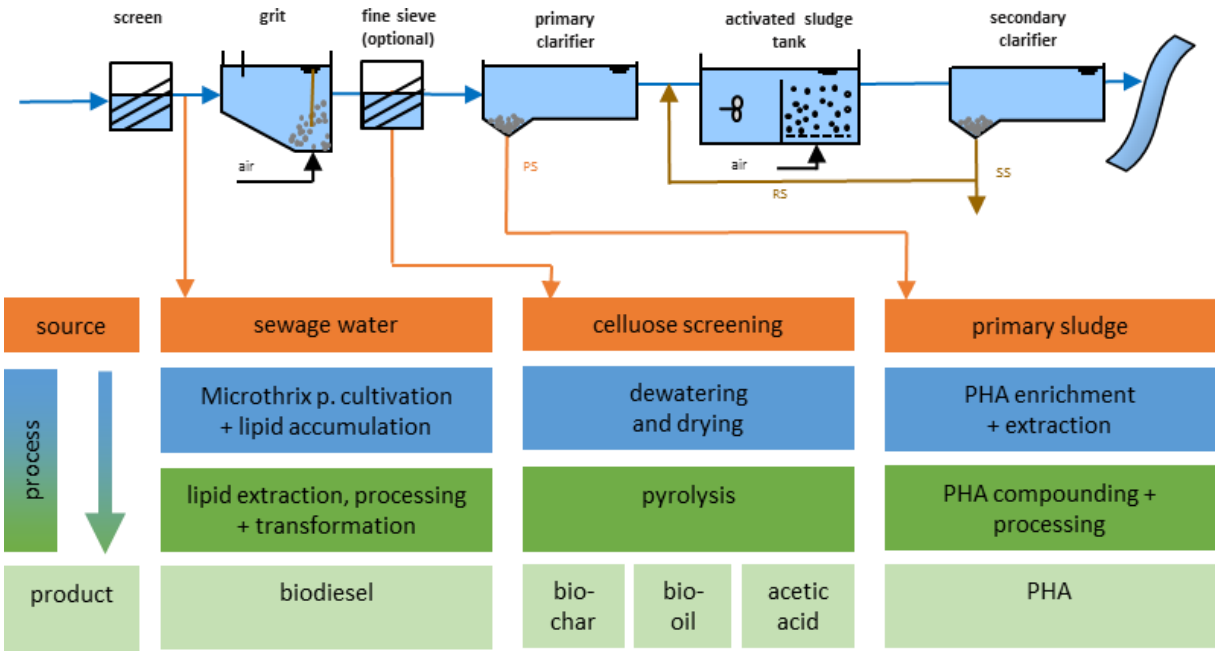


Figure 1: ***Recovery of carbon based elements from sewage in WoW!***

One of the main activities of the project was to demonstrate the technical feasibility of these three value chains in three pilots with a focus on optimisation of the different recovery and upcycling techniques and tailoring the products to market needs.

This report focusses on the activities of REMONDS Aqua Industrie GmbH & Co. KG within the Interreg North-West Europe project WOW!, with the focus on processing the activated sludge obtained from the operation of

demo scale selector for lipids (University of Luxembourg) installed at the SIVOM de l'Alzette wastewater treatment plant. The scientific results explained in this report were obtained throughout the initial preparation, concentration and drying process of the sludge, followed by lipids extraction, recovery, purificationa and production of demo-biodiesel.

2 Evaluating experiments: Concentration developments and possible approaches

2.1 Concentration tests with Sewage sludge samples

The aim of the experiments was to determine the possibility to pre-concentrate the Sewage sludge samples. We received 4 samples: I.21.0214 (31.05.2021 R1), I.21.0215 (31.05.2021 R2), I.21.0216 (31.05.2021 SVB), I.21.0217 (31.05.2021 SVV-Foam). For this purpose, filtration and centrifugation tests were carried out. In Figure 2 the original samples were shown.

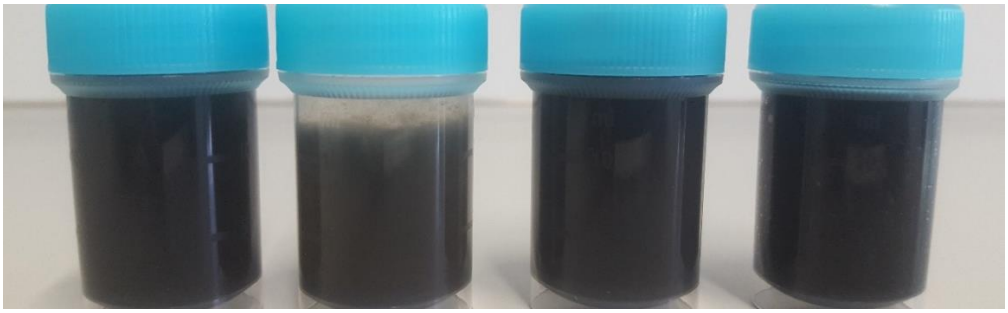


Figure 2: Original samples I.21.0214 (31.05.2021 R1), I.21.0215 (31.05.2021 R2), I.21.0216 (31.05.2021 SVB), I.21.0217 (31.05.2021 SVV-Foam) (from left to right).

First pre-test was with a 125 µm sieve, the material was running very slow through the sieve and the filtrate was not clear (Figure 3).

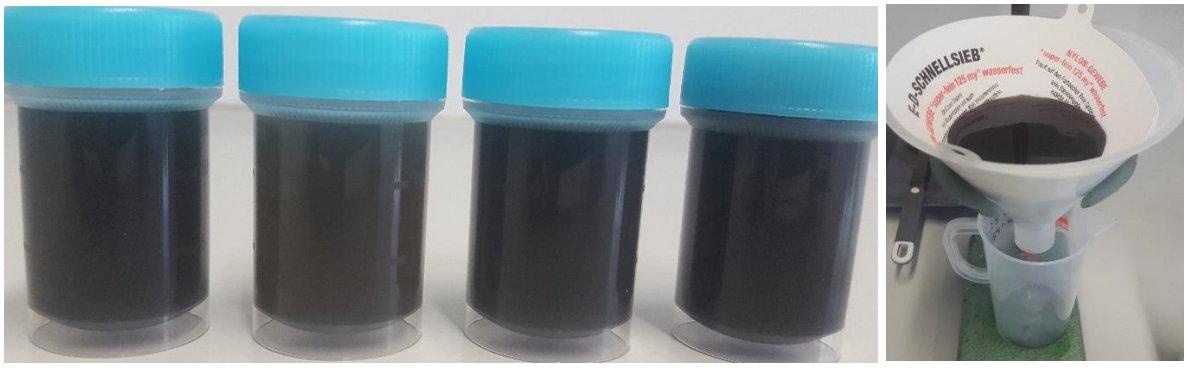


Figure 3: Sieved samples I.21.0214 (31.05.2021 R1), I.21.0215 (31.05.2021 R2), I.21.0216 (31.05.2021 SVB), I.21.0217 (31.05.2021 SVV-Foam) (from left to right). Right: Sample in the 125 µm sieve.

The filtration with depth filter was slower and not so easy for a larger volume, so the cross flow filtration would be the filtration technology to concentrate all the material (microfiltration).

Table 1: Dry matter content before and after sieving (125 µm).

Parameter	I.21.0214	I.21.0215	I.21.0216	I.21.0217
Dry matter content sample (%)	0.25	0.26	1.35	2.20
Dry matter content filtrate (%)	n.d.	n.d.	0.53	0.52

The next test was a centrifugation test, all samples were centrifuged for 5 minutes at 3000 g, the liquid supernatant was decanted and the solid phase in the centrifugation vials was weighed.

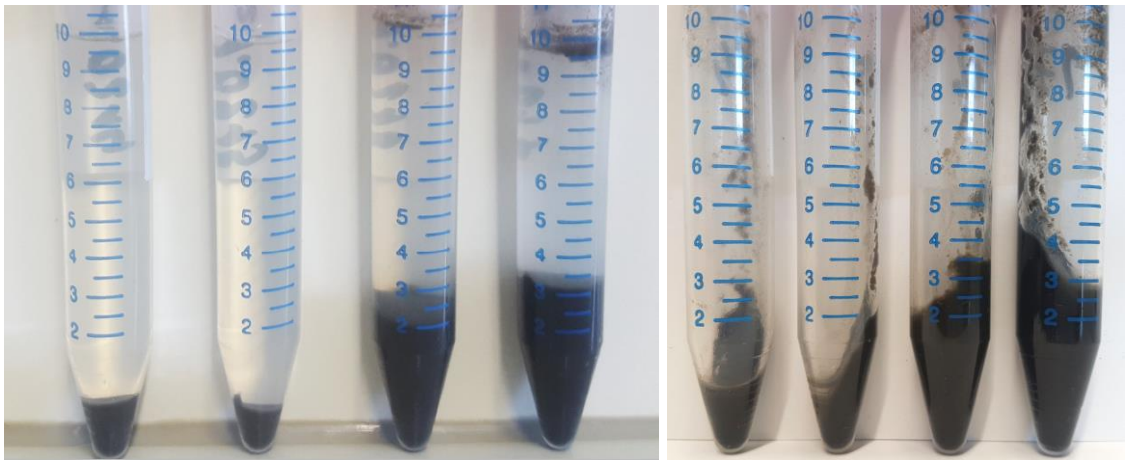


Figure 4: Centrifugation of the samples (each picture from left to right: I.21.0214 to I.21.0217). Left picture: samples after centrifugation. Right picture: solid phase after decantation of the liquid phase.

The liquid phase of the centrifugation samples was very clear and showed a very low dry matter content that was not detectable with the dry matter fast detection devices. The dry matter contents of the wet solids were measured, Table 2 shows the dry matter results and the wet and dry matter balance.

Table 2: Balance of the centrifugation test. Very low sample amounts and very low dry matter contents led to deviations in the dry matter balance.

Parameter	I.21.0214	I.21.0215	I.21.0216	I.21.0217
Dry matter content sample (%)	0.25	0.26	1.35	2.20
DM per Liter sample (g)	2.5	2.6	13.5	22.0
Wet solid per Liter sample (g)	91.9	81.3	246.9	364.5
Dry matter content solids (%)	3.45	3.11	4.83	5.52
DM in solids per Liter sample (g)	3.2	2.5	11.9	20.1
DM part loss (%)	-	2.3	11.4	8.4
DM part in solid phase (%)	-	97.7	88.6	91.6

The samples showed different contents of insoluble solids (81.3 to 364.5 g / L) according to their different dry matter content. The dry matter content of the separated solid phases was very low (3.11 to 5.52 %). Most of the

dry matter was found in the solid phase (loss during separation), the liquid phase dry matter content was not measurable. Due to the small test size the results may show higher deviations, for I.21.0214 the dry matter in the solid phase was measured higher than the total sample dry matter (3.2 g vs 2.5 g).

2.2 Concentration of Sewage sludge samples in Liter scale

Centrifugation

In order to obtain material with appreciable dry matter content for extraction tests, samples 31.05.2021 SVB and 31.05.2021 SVV Foam were dewatered by centrifugation. The materials were centrifuged with a 4x 1 Liter system at 3500 x g for 10 min and the aqueous supernatant was decanted and discarded. The sediments obtained had a pasty but not shape-retaining consistency with a dry matter content of 6.1% and 5.8% (see Figure 11). To further increase the dry matter content, the sediments were prepared for freeze-drying.

Figure 5: Sediments of the materials *31.05.2021 SVB* (on the left) and *31.05.2021 SVV Foam* (on the right) obtained by centrifugation. Prepared for freezing for freeze-drying.

Vacuum concentration

For the two materials 31.05.2021 R1 & R2 vacuum distillation using a rotary evaporator was chosen to concentrate the dry matter content. The water was extracted at 60°C and an absolute pressure of 80 mbar until the material was reduced to approx. 10 - 15 % of the initial volume. The dry substance contents obtained in this way were still a maximum of 2.6 %. For this reason, the concentrates obtained were pooled and the pool was further constricted until the evaporation of the water subsided. Thus, a pool-concentrate with a dry matter content

of 5.6 % was obtained. The pool concentrate obtained was also prepared for freeze-drying to maximize the dry matter content.

Figure 6: Material 31.05.2021 R1 during vacuum distillation. Strong foaming during reduction of pressure.

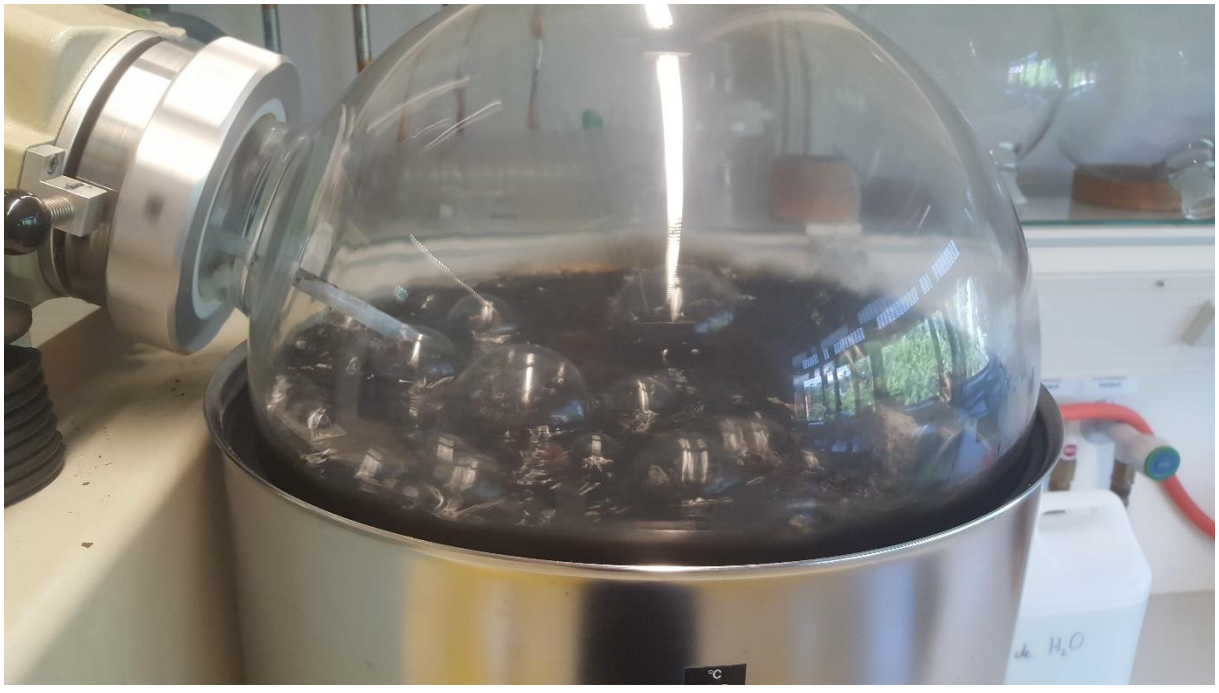


Figure 7: Material 31.05.2021 R1 during vacuum distillation. Strong foaming completely gone after degassing of the material.

Figure 8: Obtained concentrate of *31.05.2021 R1*.

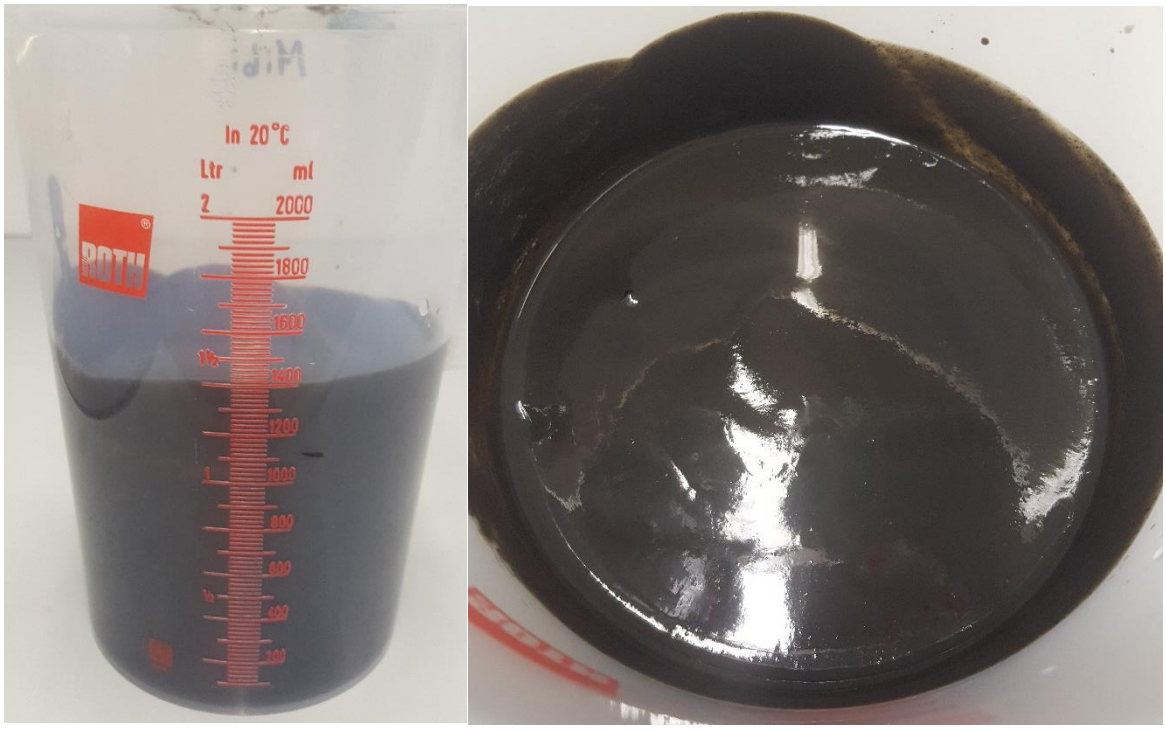
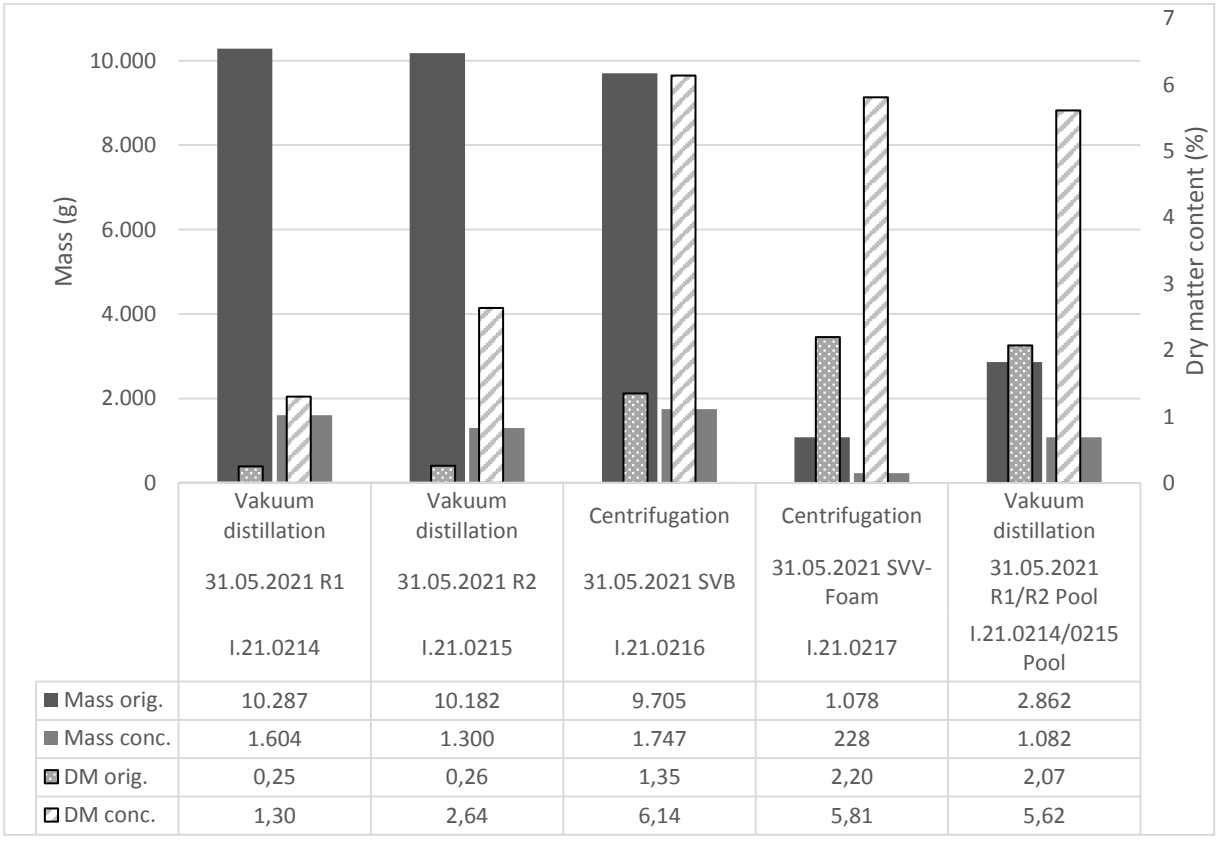


Figure 9: Obtained concentrate of *31.05.2021 R1 & R2*.

Figure 10: 31.05.2021 R1 & R2 pool-concentrate prepared for freeze drying.



	Vakuum distillation 31.05.2021 R1 I.21.0214	Vakuum distillation 31.05.2021 R2 I.21.0215	Centrifugation 31.05.2021 SVB I.21.0216	Centrifugation 31.05.2021 SVV-Foam I.21.0217	Vakuum distillation 31.05.2021 R1/R2 Pool I.21.0214/0215 Pool
Mass orig.	10.287	10.182	9.705	1.078	2.862
Mass conc.	1.604	1.300	1.747	228	1.082
DM orig.	0,25	0,26	1,35	2,20	2,07
DM conc.	1,30	2,64	6,14	5,81	5,62

Figure 11: Initial and final weights as well as initial and achieved dry matter contents.

The sediment material from vacuum distillation (I.21.0214_15 Pool) and from centrifugation (I.21.0216 and I.21.0217) were dried in a freeze dryer (Figure 12).

Figure 12: Freeze dried sediments from vacuum distillation and centrifugation.

The freeze-dried products were analysed for their composition. The results are shown in. Sample I.21.0217 GT was not analysed because it is identical to Sample I.21.0216 GT.

Table 3: Compositon of freeze dried Sewage sludge samples. Results in brackets are per dry matter (DM).

Sample	Dry matter	Protein	Fat	Minerals
I.21.0214_15 Pool GT	96.3	34.6 (36.0)	6.70 (6.96)	27.3 (28.4)
I.21.0216 Sedi GT	97.2	45.5 (46.8)	8.90 (9.16)	19.7 (20.2)

3 Initial evaluations on extraction of a fat sample from freeze dried sewage sludge

For obtaining fat samples, both freeze-dried sewage sludge samples had to be used because of the small sample quantities. Thus, 50 g of I.21.0216 Sedi GT was mixed with 500 ml of ethyl acetate and 49 g of I.21.0214_15 Pool GT with 490 ml of 2-propanol. (Figure 13).

Figure 13: Extraction of fat from freeze-dried sewage slug.

The extractions then took place for 1 hour at 50 °C in a rotary evaporator under low vacuum for ethyl acetate. Extraction with 2-propanol was also terminated after one hour, the separated sediment was mixed with another 490 ml of 2-propanol after filtration and the extraction was continued under the same conditions for another 3h. The residual sediment was separated from the supernatant by filtration through pleated filters.

The solvents were then removed from the obtained supernatants in a rotary evaporator. A small amount of fat then remained in each case, which was balanced.

Figure 14 shows the results of the fat extractions with respect to the solvent used and the extraction time.

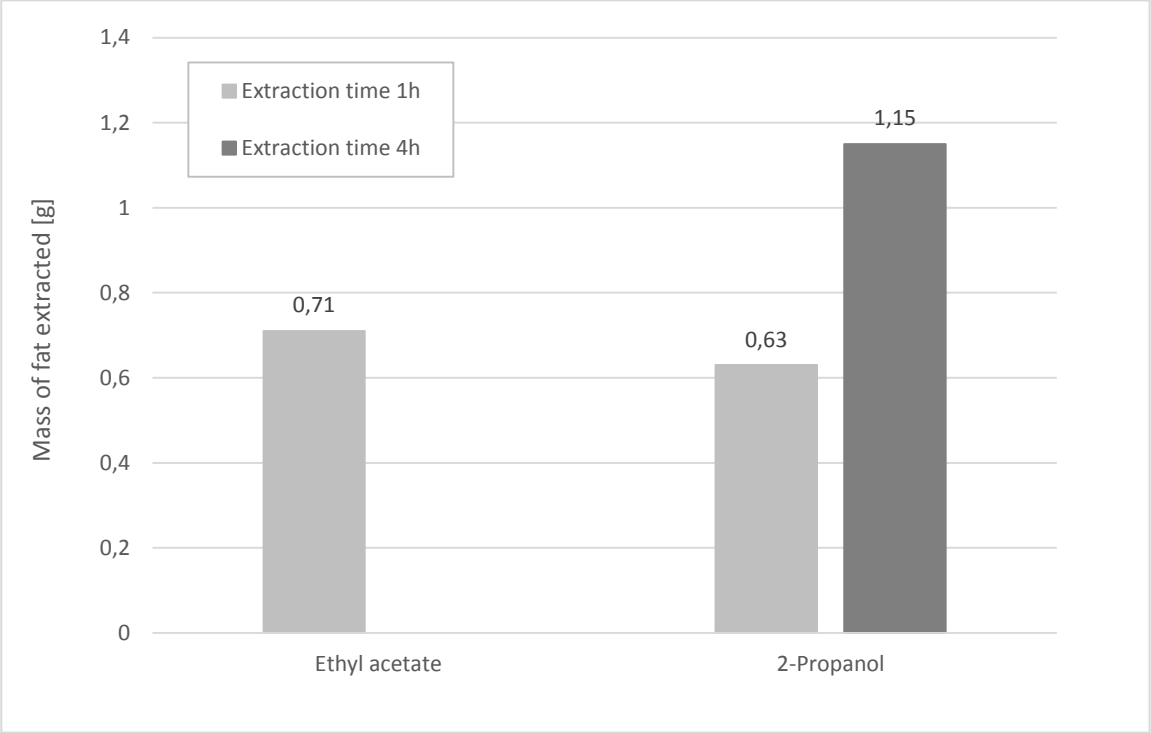


Figure 14: Extracted fat masses from freeze-dried sewage sludge with solvents.

Using the maximum amount of fat possible for each sample used, a yield can then be calculated for each extraction (Table 4).

Table 4: Calculation of the yields of the solvent fat extractions.

Material	Solvent	Ext. Time [h]	Fat max. [g]	Fat ext. [g]	Yield [%]
I.21.0213_14	2-propanol	1	3.41	0.63	18.5
		4	3.41	1.15	33.7
I.21.0216	Ethyl acetate	1	4.58	0.71	15.5

The results show that a longer extraction time can significantly increase the yield of fat. Problematic chemicals can be avoided here, and 2-propanol can be used and reused as the solvent.

4 Concentration and drying of the main activated sludge sample

4.1 Raw material

The collected activated sludge from University of Luxembourg was delivered to ANiMOX on 15.09.2021 in 6 barrels/buckets (see Figure 15) and had a gross mass of 162.15 kg.

Figure 15: Collected activated sludge from University of Luxembourg.

The information about the six samples is collected in Table 5 and Table 6.

Table 5: Delivery information of the 6 sample materials

AX-No	Delivery date	Gross weight	Description	Collection on	Source
I.21.0278	15.09.2021	26.6 kg	Activated Sludge (Foam I)	25.08.2021	Uni Luxemb.
I.21.0279	15.09.2021	22.8 kg	Activated Sludge (Foam II)	31.08.2021	Uni Luxemb.
I.21.0280	15.09.2021	27.4 kg	Activated Sludge (Foam III)	07.09.2021	Uni Luxemb.
I.21.0281	15.09.2021	25.8 kg	Activated Sludge (Foam IV)	07.09.2021	Uni Luxemb.
I.21.0282	15.09.2021	31.5 kg	Activated Sludge (Mixed Liquor V)	07.09.2021	Uni Luxemb.
I.21.0283	15.09.2021	28.1 kg	Activated Sludge (Foam VI)	08.08.2021	Uni Luxemb.

After opening the materials showed different conditions, this information is collected in Table 6 and a picture of the foam and the liquid material is shown in Figure 16.

Table 6: Amount and condition of the delivered materials.

AX-No	Empty [kg]	Gross Weight [kg]	Net Weight [kg]	Description	Material
I.21.0278	1.04	26.55	25.51	Foam I	Stable foam
I.21.0279	1.66	22.80	21.14	Foam II	Stable foam
I.21.0280	1.66	27.40	25.74	Foam III	Stable foam
I.21.0281	1.66	25.80	24.14	Foam IV	Stable foam
I.21.0282	1.66	31.50	29.84	Mixed Liquor V	Liquid sludge
I.21.0283	1.04	28.10	27.06	Foam VI	Stable foam
total	8.72	162.15	153.43		

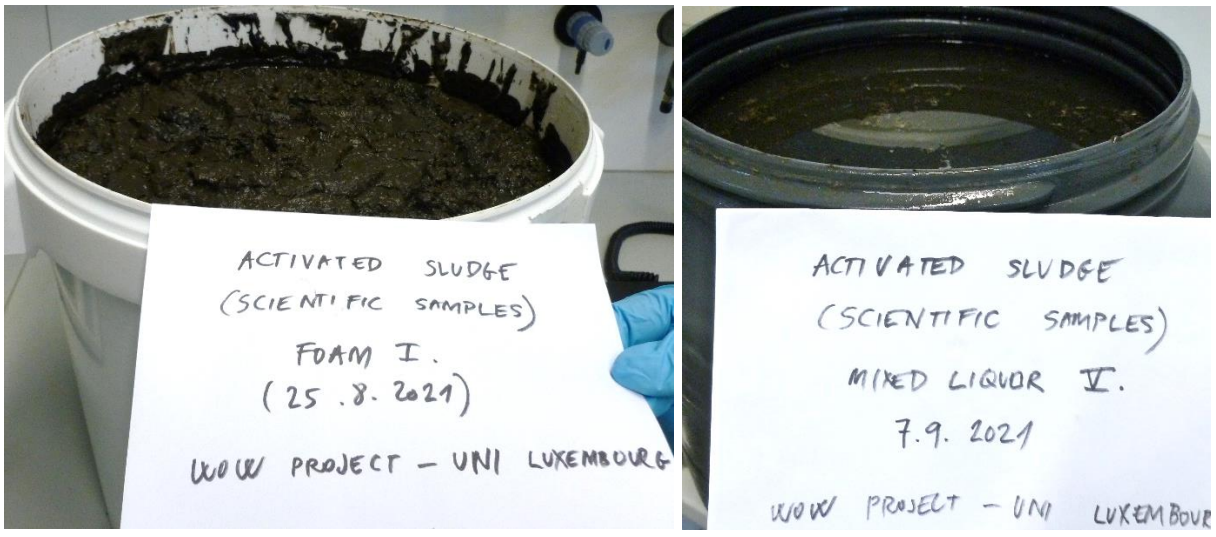


Figure 16: Comparison of the materials stable foam (left) and liquid sludge (right)

After registration of the materials, the materials were directly tested for the concentration step. The results are shown in the following chapter.

4.2 Concentration of the material

4.2.1 Direct separation of activated sludge

After receiving the samples, the direct concentration through centrifugation at 4000 rpm, 10 min was tested. The aim was to concentrate the material to a dry matter of 10-20 % to freeze or air dry the material. The first test was with the material V mixed liquor (Figure 16). With this material was a good separation possible. Table 7 show the results and Figure 17 a picture after centrifugation.

Table 7: Results of the separation of the material V mixed liquor.

Parameter	Supernatant [g]	Sediment [g]	Total mass [g]
Wet weight [g]	23347,1	6219,1	29566,2
Dried material [g]	21,0	448,3	469,3
Dry matter [%]	95,58	96,70	96,69
Dry mass [g]	20,1	433,5	453,8
DM of Wet calc [%]	0,09	6,97	1,53

Figure 17: Material V mixed liquor before and after centrifugation.

The other materials were not directly separable, only a supernatant of 5 % was reached. Therefore, literature studies about the concentration of sewage sludge were made. The large machines for the concentration were not applicable because of the low amount, heat treatment with over 100 °C could have an influence on the fatty acid composition, but there was also a possibility to reduce the sewage sludge foam stability by freezing the material. The results are shown in chapter 4.2.2.

4.2.2 Freezing/Thawing and separation of activated sludge

To destroy the sewage sludge foam stability, one barrel at a time was frozen for at least 3 days, and then thawed again. After thawing the material was more liquid like the mixed liquor and the solid material was easy separable

by centrifugation at 4000 rpm, 10 min. Figure 18 shows the raw material before and after freezing and Figure 19 the material after centrifugation and separation of the fractions.

Figure 18: Activated sludge-foam before (left) and after freezing (right).

Figure 19: Activated sludge-foam in beaker for centrifugation (bottom of left picture), after centrifugation with liquid phase (top of left picture) and solid phase (right).

With this method the solids and the liquid part could be separated in the 5 other buckets. After separation the solid material was used for freeze-drying and air drying and the liquid material was used for concentration (chapter 4.2.3) and freeze drying.

The results are shown in Table 8 to Table 12.

Table 8: Results of the separation of the material I foam I.

Parameter	Supernatant [g]	Sediment [g]	Total mass [g]
Wet weight [g]	12883.1	6184.1	25467.2*
Dried material [g]	21.5	1598.4	1619.9
Dry matter [%]	96.86	94.04	94.07
Dry mass [g]	20.8	1503.1	1523.8
DM of Wet calc [%]	0.16	18.1	5.98

*6400 g was dried separately before centrifugation

Table 9: Results of the separation of the material II foam II.

Parameter	Supernatant [g]	Sediment [g]	Total mass [g]
Wet weight [g]	13972.6	7279.9	21252.5
Dried material [g]	23.5	1336	1359.5
Dry matter [%]	96.89	98.94	98.92
Dry mass [g]	22.8	1321.8	1344.8
DM of Wet calc [%]	0.16	18.2	6.33

Table 10: Results of the separation of the material III foam III.

Parameter	Supernatant [g]	Sediment [g]	Total mass [g]
Wet weight [g]	16895.4	8521.0	25416.4
Dried material [g]	31.9	1486.3	1518.2
Dry matter [%]	96.75	97.09	97.09
Dry mass [g]	30.9	1443.1	1474.0
DM of Wet calc [%]	0.18	16.9	5.80

In Figure 20 the separation of Foam II after freezing is shown with a dry and not so wet sediment fraction like with sediment V and VI.

Figure 20: Separated Foam III material with sediment (left) and liquid (right)

Table 11: Results of the separation of the material IV foam IV.

Parameter	Supernatant [g]	Sediment [g]	Total mass [g]
Wet weight [g]	16283.8	6438.3	24132.1*
Dried material [g]	32.2	1150.8	1183.0
Dry matter [%]	97.10	98.23	98.22
Dry mass [g]	31.3	1130.4	1161.9
DM of Wet calc [%]	0.19	16.5	4.81

*1410 g was dried separately before centrifugation

Table 12: Results of the separation of the material VI foam VI.

Parameter	Supernatant [g]	Sediment [g]	Total mass [g]
Wet weight [g]	7871.4	14134.5	27005.9*
Dried material [g]	10.3	1021.9	1032.2
Dry matter [%]	87.89	95.21	95.14
Dry mass [g]	9.05	973.0	982.0
DM of Wet calc [%]	0.12	5.60	3.64

*5000 g was dried separately before centrifugation

The dry matter of the sediment was suitable for drying at 16-18 % in materials I – IV, in material VI the dry matter was lower, because of a worse separation, but drying was also possible.

4.2.3 Concentration of separation-supernatants of activated sludge

The supernatant of the centrifugation steps showed with 0.1 - 0.2 % a really low dry matter. However, to recover possible residual fat, the liquid was concentrated to 0.5 - 1 L for the freeze-drying (Figure 21).

Figure 21: Concentration of the supernatant in a vacuum evaporator.

A total of 91.2 liters of supernatant was concentrated over several days, each material to a separate concentrate for the drying step.

4.3 Drying of concentrated activated sludge

4.3.1 Freeze drying of concentrated activated sludge

The activated sludge was collected on plates, and frozen at -25 °C. In the freeze dryer 5 kg material could be dried in one step. Five freeze drying steps were made with 5 kg material each. In Figure 22 the steps for freeze drying concentrated sludge are shown.

Figure 22: Concentrated sludge wet (left top) and dry (right top) sediment for freezing on plates, freeze drying (bottom left) and freeze-dried samples (bottom right).

The freeze-drying process took app. 3-4 days for drying one batch and leads to a fine material which is easy to homogenize.

4.3.2 Air drying of concentrated activated sludge

The rest of the concentrated sludge (app. 24 kg) was dried with an air dryer oven at 80°C. the material was placed on plates, dried in the oven and turned twice a day. The drying time was app. 3 days. In Figure 23 the drying process in the air dryer is shown.

Figure 23: Activated sludge on a drying plate (top), Air drying oven (middle left), dried material (middle right and bottom).

In total, a quantity of 1.3 kg dry material was obtained in the drying oven from approx. 24 kg of concentrated sludge. The material was hard and grainy and difficult to homogenize.

4.3.3 Freeze drying of concentrated activated sludge supernatant

After the concentration step an amount of app. 6 L supernatant concentrate had to be dried. In two freeze-drying runs the material was dried on separate plates. In Figure 24 the concentrated Material and the freeze-dried material is shown.

Figure 24: Concentrated and freeze-dried material of the supernatants.

After freeze drying the material was fine, easy to homogenize and beige to brown.

4.4 Summary of the concentrating experiments

Within the project work all delivered material were processed through various pre-treatment, concentration and drying steps to a dry material for extraction of fat. In Table 13 all delivered samples are shown.

Table 13: Products from the drying process (FD = Freeze dried)

Starting material	Sample	Sample vessels	Mass (g)
I.21.0278 (Foam I)	Liquid (FD + 80°C)	1	21.5
	Solid (80°C)	3	1317.2
	Solid (FD)	1	281.2
I.21.0279 (Foam II)	Liquid (FD + 80°C)	1	23.5
	Solid (80°C)	2	1336.0
I.21.0280 (Foam III)	Liquid (FD + 80°C)	1	31.9
	Solid (80°C)	2	1486.3
I.21.0281 (Foam IV)	Liquid (FD + 80°C)	1	32.2
	Solid (80°C)	1	253.5
	Solid (FD)	4	897.3
I.21.0282 (Mixed Liquer)	Liquid (FD + 80°C)	1	21.0
	Solid (FD)	2	448.3
I.21.0283 (Foam VI)	Liquid (FD + 80°C)	2	10.3
	Solid (80°C)	2	879.0
	Solid (FD + 80°C)	1	142.9
Total	Liquid (FD + 80°C)		140.4
	Solid (80°C)		5720.3
	Solid (FD + 80°C)		1321.4

In a total processing time of 1.5 months, the material of 153 kg sludge was concentrated and dried to approximately 6.2 kg products for the fat extraction. The typical sample dry matter was between 94 and 98 %.

5 Study of sludge and recovery of lipids

5.1 Characterization of sludge

The first analysis conducted on the sludge were related to a preliminary characterization. The determination of the residual humidity, the mineral components (ashes) and the relevant metal profile were determined. 4-5 g of sludge were kept in an oven at 105°C for 24 h. 10% of their initial weight was lost as residual humidity. Dried sludge was then calcined at 550°C for three hours. Ashes contents were determined (which goes around 24-26% of initial total solids, TS), solubilized and analyzed to obtain the metal profile (please see Table 14).

Table 14: Metal composition determined on the ashes and referred to the initial dried solid. A very similar profile was always determined analyzing the different sludge.

Metal	Value	Unit
Na	1,17689	%TS
Mg	0,59895	%TS
Al	1,03821	%TS
K	1,11366	%TS
Ca	5,98789	%TS
V	0,00473	%TS
Cr	0,01204	%TS
Mn	0,02910	%TS
Fe	2,64829	%TS
Co	0,00048	%TS
Ni	0,01228	%TS
Cu	0,03400	%TS
Zn	0,25964	%TS
As	0,00079	%TS
Se	0,00038	%TS
Cd	0,00102	%TS
Sn	0,00242	%TS
Ba	0,06230	%TS
Pb	0,01640	%TS

Figure 25: Ashes obtained from sample I.21.0278 FOAM 1 Solid FD.

Figure 25 shows how the ashes obtained from these samples appeared. These reddish powders, for the presence of relatively high content of Iron, contained also significant amount of sodium, potassium, aluminium, magnesium and calcium.

5.2 Determination and recovery of total lipids from sludge

The estimation of the total lipid content was carried out using three different methods:

1. The *Bligh and Dyer* method;
2. The in-situ analysis carried out with methanol and HCl for the gas-chromatographic determination of Fatty Acids (free and not);

The first method adopted for the recovery of lipids from sludge was an adapted version of the Bligh and Dyer method. $CHCl_3$:MeOH 1:1 was used to extract the lipid phase. Three different phases were detected: an upper methanolic solution, a bottom-heavy phase where most of lipids were dissolved in (Chloroform) and a solid which was in the interface (please see Figure 26). This method is the most conservative approach to overall recover lipids from a biomass and allow the maximum lipid extract to be achieved.

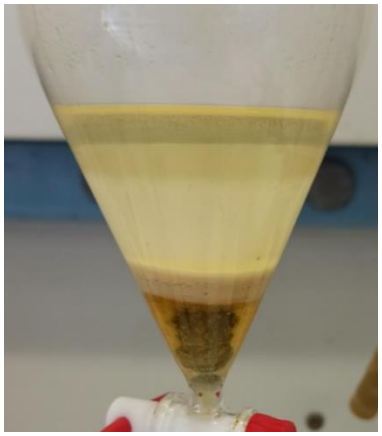


Figure 26: Separation of phases after the Bligh and Dyer method extraction.

The present method was applied on four different samples, namely I.21.0278 FOAM 1 Solid FD, I.21.0279 FOAM 2 Solid 80°, I.21.0280 FOAM 3 Solid 80° and I.21.0281 FOAM 4 Solid FD.

The overall extracts were around 6.85-7.14 wt.%$_{TS}$ (lipids yield). The interfaces were accounted to represent 0.5-1% of the initial solids. Both were analysed directly through FTIR (please see Figure 27 and Figure 28).

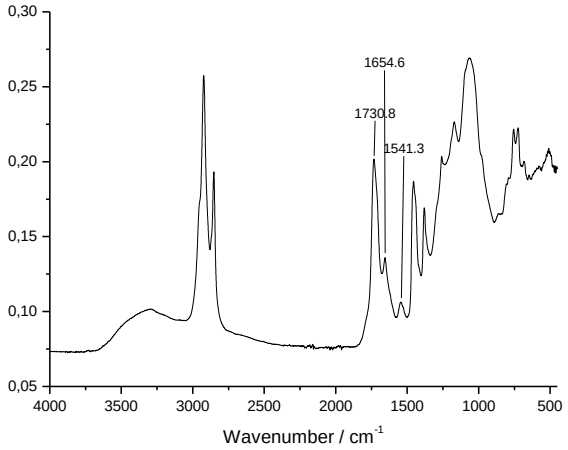


Figure 27: Example of FTIR analysis on the soluble component derived from the Bligh and Dyer extraction.

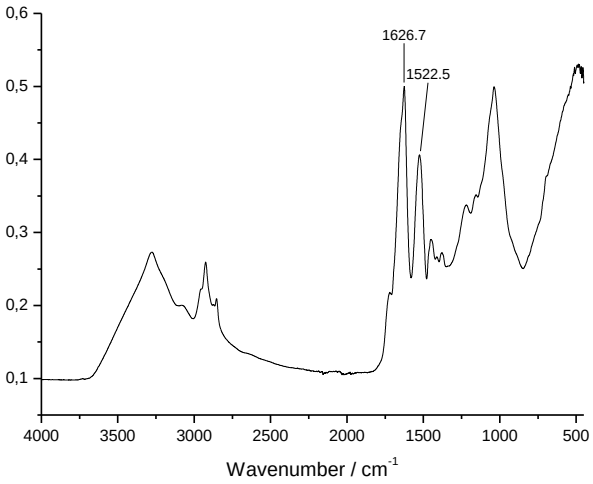


Figure 28: FTIR spectrum of solids recovered at the interface from the Bligh and Dyer extraction.

The direct gas-chromatographic analysis of these extracts evidenced that no FAMEs were detected. However, the signal at 1730 cm^{-1} in Figure 27 evidenced the presence of esters, most probably heavy esters (waxes of sterols and fatty alcohols). In addition, the signal located at 1680-1700 cm^{-1} clearly evidenced the presence of carboxylic acids.

These extracts were then reacted for being gas-chromatographically analyzed for the quantification of FFAs and fatty acids heavy esters as methyl esters. 15% of the extracted lipids were FFAs, while heavy esters were accounted for further 15%. These determinations allow the overall amount of biodiesel achievable from these samples of sludge to be determined. In the best case, around 1.6-2% of the initial sludge could be converted to FAMEs (biodiesel yield).

After this preliminary investigation a more methodological analysis was run on the different samples.

Residual humidity, ashes content and total potential FAMEs were determined on samples of sludge received (see Table 15). In addition, the elaboration of the gas-chromatographic analysis, allowed the fatty acid profiles, the percentage of unsaturated esters and the average molecular weight (AMW) to be determined.

Table 15: Extraction Results

Entry	Code	Description	State		Weight	FAMEs[1] (Biodeisel Yield)	AMW	u	Total Solids	Ashes	Acid/Sludge/MeOH	Extracts (Biofuel Yield)	FAMEs from FFAs*	t FAMEs**	Acid
					(g)	(mg/g_{ST})	(uma)	(%)	(%)	(%)	(g/g/g)	(wt. %)	(%)	(%)	
1	I.21.0278	FOAM 1	S	FD	277,8	18,1	226,64	34,80	95,4	24,9	40/300/700	6,53[b]	17,4	30,4	H_2SO_4
2	I.21.0278	FOAM 1	L	FD+80°	18,9	4,3									
3	I.21.0278	FOAM 1	S	80°	709,4	17,6	218,61	26,20	97,7	26	50/700/400	**14,26[a]/1,85** [b]		8-32	H_2SO_4
4	I.21.0278	FOAM 1	S	80°	592,5	14,6	235,57	38,76	95,9	24,6	44/580/500	5,2 [b]	12,08	25,5	H_2SO_4
5	I.21.0279	FOAM 2	L	FD+80°	23	7,1									
6	I.21.0279	FOAM 2	S	80°	714,6	10,4	233,92	37,18	97,1	26,4	50/350/450	5,26 [b]	14,5/17,2	16/21,6	HCl/ H_2SO_4
7	I.21.0279	FOAM 2	S	80°	606,6	9,6	234,71	37,96	97,9	24,89	40/600/500	4,7 [b]	11,98	28,17	H_2SO_4
8	I.21.0280	FOAM 3	L	FD+80°	28,9	2,1									
9	I.21.0280	FOAM 3	S	80°	744,4	24,0	225,22	27,09	94,6	25,75	50/700/400	**14,68** [a] **/2,7** [b]		9-25%	HCl
10	I.21.0280	FOAM 3	S	80°	727	11,5	244,16	37,42	93,6	26,4	50/350/500	**20,8** [a] **/20,6** [a]	10,46	10,5	H_2SO_4/HCl
11	I.21.0281	FOAM 4	S	FD	240,5	18,0	246,89	37,53							
12	I.21.0281	FOAM 4	S	FD	214,3	16,9	243,99	42,64	95,2	26,2	30/300/300	4,49 [b]	12	30	HCl
13	I.21.0281	FOAM 4	S	FD	224,4	20,2	244,64	42,45							
14	I.21.0281	FOAM 4	S	FD	211,3	21,5	245,31	44,07							
15	I.21.0281	FOAM 4	S	80°	237,5	20,4	225,51	32,74	94,7	25,9	50/350/450	5,15 [b]	16,4	28,2	H_2SO_4
16	I.21.0281	FOAM 4	L	FD+80°	29,1	<dl									
17	I.21.0282	Mixed liquor	L	FD+80°	18,7	1,1									
18	I.21.0282	Mixed liquor	S	FD	104,5	21,0	224,33	33,49	93,9	24,8	50/350/450	5,4 [b]	15,7	27,3	H_2SO_4
19	I.21.0282	Mixed liquor	S	FD	339,8	22,5	250,57	40,02	95,4	26,4	50/350/450	5,3 [b]	13,4	25,2	H_2SO_4
20	I.21.0283	FOAM VI	L	FD+80°	9,2	3,2									
21	I.21.0283	FOAM VI	S	FD+80°	139,4	17,5	227,96	34,12	96,3	25,5	50/350/450	5,2 [b]	14,7	26	H_2SO_4
22	I.21.0283	FOAM VI	S	80°	258,7	18,0	219,98	36,05	93,13	25,7	26/250/500	5,96 [b]	13,6	28,90%	H_2SO_4
23	I.21.0283	FOAM VI	S	80°	632,2	12,7	245,37	45,98	91,46	27,3	0/500	2,8 [b]	nd	30,14	No Acids

S: Solid; L: Liquid; FD: Frieze dried; AMW: Average Molecular Weight; u: Unsaturations;

[1]: total potential FAMEs determined on dried sludge;

[a]: total residue obtained from the evaporation of the methanol

[b]: total residue obtained from the evaporation of the hexane

*: FAMEs from FFAs evaluated on the lipid extract

**: t FAMEs, total FAMEs determined on the lipid extract

The residual humidity was very similar, even using different drying approach. The total potential FAMEs on dried sludge and the relevant profile of fatty acids was also redundant. Figure 29 shows an example of the final profile.

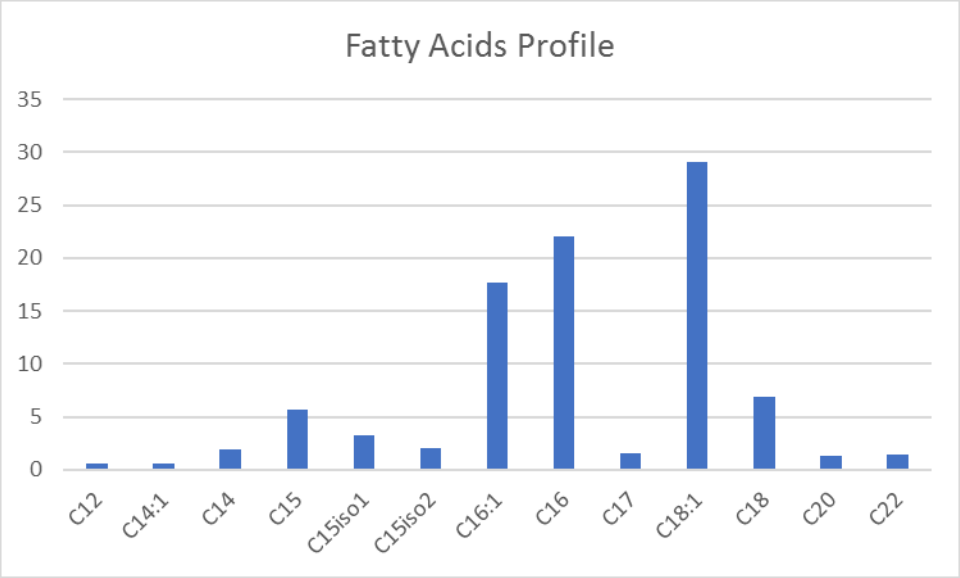


Figure 29: Fatty acids profile detected from sample I.21.0278 FOAM 1 Solid FD.

The presence of C15 (different isomers) and C16:1 evidenced a clear provinience from the bacterial feedstock. According to the data reported in Table 15, AMW (234,90±16 uma) and the total unsaturated fatty acids (36,97±9%) were almost the same in all the samples. The ashes content (25,77±1,5%) was also very similar for each samples.

After this initial characterization of sludge, the study was focused on the extraction of the lipid component. Considering that samples obtained from liquid (red entries in the Table 15) contained low amount of lipids, this phase of the study was conducted on the solid samples only, according the scheme of operation reported in Figure 30.

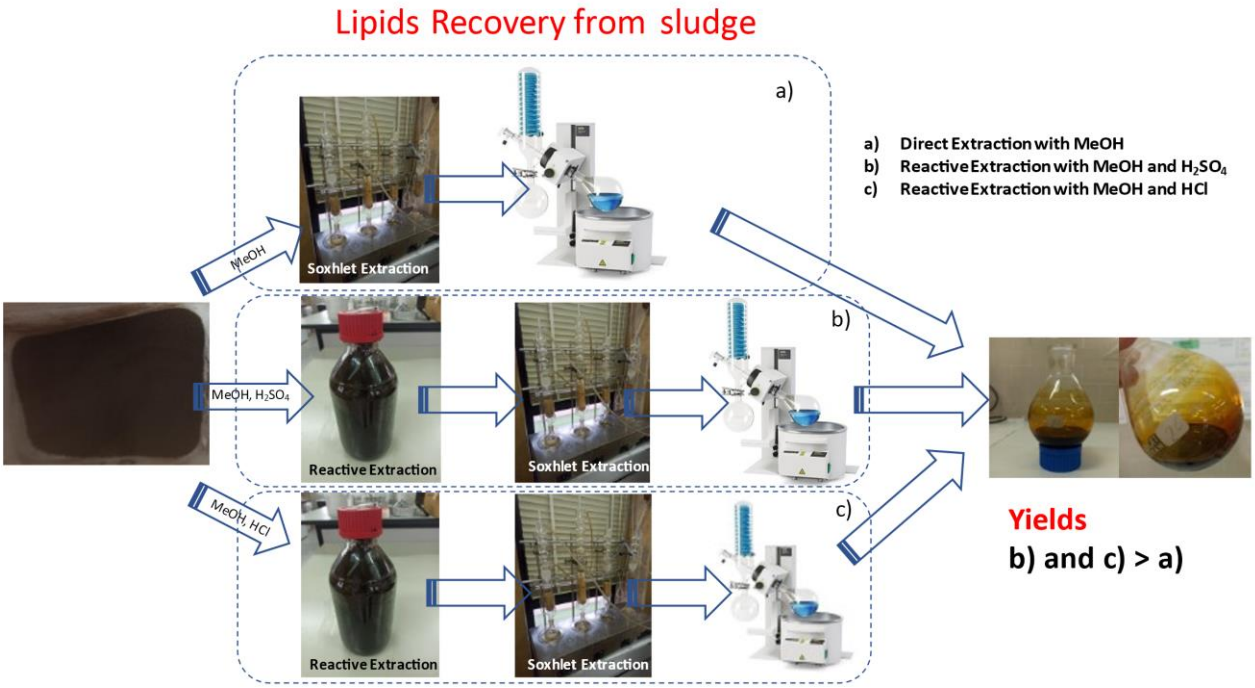


Figure 30: Lipids recovery from sludge. Three different routes were tested: a) the direct extraction from dried sludge using methanol, the extraction operated with methanol on a pretreated sludge using b) H_2SO_4 and c) HCl.

In detail, the following tests were operated:

1. an extraction through Soxhlet technique using methanol directly on the dried sludge (Entry 23, Table 15);
2. an extraction through Soxhlet technique using methanol after a preliminary treatment with a mineral acid (HCl and H_2SO_4) and methanol on dried sludge.

In general, the initial pretreatment with mineral acids (50°C, 24 h), allow the lipid extraction to be more efficiently realized. In fact, the composition of the extract was monitored through gas-chromatographic analysis and qualitatively studied through FTIR (please see Figure 31).

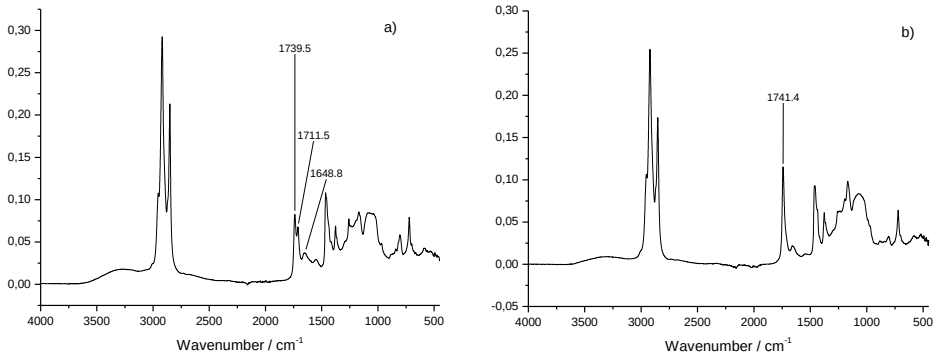


Figure 31: a) FTIR spectra related to methanol extract (Entry 23, Table 15); b) FTIR spectra recorded on the extract obtained from an acid pretreatment (Entry 6, Table 15).

The gas-chromatographic analysis of the extract obtained from the direct extraction with methanol evidenced a total absence of methyl esters of fatty acids, confirming that the signal ester at 1739,1 cm^{-1} was due to fatty esters initially present in the sludge. Besides, the peak at 1711 cm^{-1} can be attributed to FFAs. As for the extract obtained from acid pretreatment, the peak at 1741 cm^{-1} can be attributed to FAMEs directly obtained during the pretreatment.

The effect of the amount of acids was also tested. Considering that a relatively high content of sodium, potassium, magnesium, iron and calcium salts are present in the initial sludge, the addition of acid suffered from the formation of buffered system. When the pretreatment was operated with a sufficient excess of acid, up to obtain a final acid solution (entries 6 and 10 in Table 15), besides an efficient recovery of lipid extract, a high methyl esters of fatty acids content was obtained. This result can be explained by the occurrence of the transesterification of fatty esters in the pretreatment step. On the contrary, when the pretreatment was conducted with less acid, the extraction of lipids remained efficient, but only the direct esterification of FFAs was obtained. This required a further transesterification step which was operated under acid or alkaline conditions, using toluene as a cosolvent.

In any case, the extract obtained by evaporating the methanol used for the extractive step contained several other compounds not associable to FAMEs. To reduce the presence of these contaminants, a solvent-solvent extraction using hexane vs the methanolic solution was operated. Usually, only one fourth of the initial extract was collected into the hexane phase, obtaining a biofuel with a final concentration of FAMEs (total) of about 30%.

5.3 Conclusions of the study of sludge and recovery of lipids

Different sludge were characterized and results were compared. Their composition was reproducible, and this aspect represent a positive point since a standard product could be eventually obtained.

The best route to extract lipid from sludge was studied. In detail, considering that the total content of grease therein was very limited, a solventless approach was not applicable. For this reason, the most conservative approach was adopted. Sludge were firstly dried (different drying strategies were tested, namely freeze dry, the thermal evaporation (80°C) and a combination of these), followed by a solvent extraction. It was verified that no effects were observed by changing the drying procedure on the composition of the lipid (FFAs profile) and their overall content.

Once that dried sludge were obtained, different extracting approaches using methanol as a solvent were tested. The direct extraction with methanol on dried sludge did not produce efficient results. A pretreatment with mineral acid (HCl and/or H_2SO_4) was beneficial for the reaction of formation of FAMEs (in-situ reaction of FFAs and fatty esters) and for the concomitant recovery. The use of methanol was effective in extracting lipids, but it was not selective. A very high presence of contaminants was also ascertained: the final content of FAMEs into the raw extract was not bigger than the 10%. To concentrate this FAMEs content an extraction with hexane was tested on the methanol extract. This operation allowed a biofuel to be obtained with a significant concentration of FAMEs (three times the initial concentration, to about 30%).

In the present study, the main focus was the obtainment of biodiesel, but actually several other streams can be better valorized and necessitate further investigations. For example, the methanolic exhausted phase deriving from the extractive purification operated with hexane, could be potentially a source of minerals and fatty alcohols. The correct estimation of costs/benefit ratio related to this train of technology cannot be conducted ignoring the fate of this stream.

5.4 Experimental Section 1

Sodium hydroxide (NaOH, 99%), potassium hydroxide (KOH, 85%), hydrochloric acid (HCl, 37%), sulfuric acid (H_2SO_4, 98%), diethyl ether ($(C_2H_5)_2O$, 99.5%), hexane (C_6H_{14}, 95%), methanol (CH_3OH, 99.8%) and ethanol (C_2H_5OH, 99.8%) were purchased from Carlo Erba. Chemical reagents were of analytical grade and used without any purifications or treatments.

All the gas-chromatographic experiments were conducted in triplicate, allowing the average value and the standard deviations to be obtained. The mean value for each parameter was eventually reported, with a relevant variability that did not exceed the 5%.

Sludge were preliminary milled through a ball mill (Retsch MM301 Mixer Mill, Haan, Germany) with zirconium oxide jars to reach the suitable particle size for the analysis.

Figure 32: a) example of typical sludge as received and b) after milling.

Instruments

FT-IR spectra were collected using a Nicolet Summit FT-IR Spectrometer with an ATR accessory equipped with a diamond laminate crystal. Spectra were collected from 4000 to 400 cm^{-1}, with 64 scans per spectrum and 4 cm^{-1} resolution.

Identification of FAMEs was carried out by using a Perkin Elmer Clarus 500 gas-chromatograph interfaced with a Clarus 500 spectrometer. Quantitative determinations were performed with an Agilent 8890B GC system with flame ionization (FID) and thermal conductivity (TCD) detectors. Both instruments were configured for cold on-column injections with an HP-5MS capillary column (30 m; Ø 0.32 mm; 0.25 µm film). The injector and the oven followed the same temperature program: an initial temperature of 40 °C was kept constant for 2.5 min, then was raised to 280 °C by using a 10 °C min^{-1} ramp and finally to 300 °C with 20 °C min^{-1}. The final temperature was kept constant for 15 min. Metal analysis (Na, K, Mg, Ca, Al, …) were carried out using a 7000X ICP-MS instrument (Agilent Technologies). 100 mg of sample were mineralized in 9 mL of HCl (37%), 3 mL of HNO_3 (67 %), 4 mL of H_2O_2 (50 %) for 2 h at 503 K using a Milestone START E microwave oven. The mineralized samples were solubilized into 100 mL of Milli-Q water (0.6 $\mu S^2\ m^{-2}\ \Omega$), filtered, diluted and analyzed.

Total solids and ashes

Total solids (*TS*) were determined according to the ISO 11465:1993 method. A sample of PS (100 g) was placed in an oven at 105 °C for 24 h, until obtaining a constant weight. TS were expressed as the weight of residual

solids obtained after each thermal treatment per litre of sludge (g/L). The dried sample was then heated in a muffle furnace at 550 °C for 3 h for determining ashes content (mg/g_{TS}).

Bligh and Dyer method adapted to sewage sludge

Lipid extraction from pre-treated sewage sludge was carried out according to the Bligh and Dyer protocol taking into account the difference in terms of water content between tissue for which this method was developed and sludge that was going to be analyzed. In particular, 5 g of dry sludge were homogenized with 50 mL of $CHCl_3$ and 50 mL of MeOH for 1h. The mixture was filtered and then washed two times with 10 mL of $CHCl_3$/MeOH mixture and the collected liquid was extracted with 50 mL of water by using a separatory funnel. The organic portion was then dehydrated using sodium sulphate. The solution was filtered and the volume reduced in a bath of water at 50°C. Lipid content was then determined gravimetrically after evaporating chloroform phase to dryness. Finally, the obtained oil was dissolved in 2 mL of n-hexane and analyzed by GC-MS.

Gas-chromatographic determination of FFAs and total transesterifiable lipids

About 100 mg of sample (dried sludge or extracted lipid) were placed in a vial together with 50 mg of HCl (37%) and 3 ml of methanol and 4 mg of methyl heptadecanoate used as internal standard. The reactor was closed and kept in a oven at 60°C for 2 and 24 h to determine gas-chromatographically FFAs and total transesterifiable lipids. Then, 1 µL of the supernatant was injected into the gas-chromatograph. Trans-esterifiable lipids were determined according to the Eq. 1

$$\textit{Trans-esterifiable lipids } (mg/g_{TS}) = \frac{\sum A_i}{A_S} \cdot \frac{w_S}{w_{TS}} \qquad (1)$$

Where A_i is the area of the i-th fatty acid methyl ester detected by gas-chromatography, A_S and w_S are area and weight (mg) of the internal standard methyl heptadecanoate, respectively, and w_{TS} is the amount of starting dried sludge (g). In addition, the average molecular weight (*AMW*) was calculated according to the following equation (Eq. 2):

$$AMW = \frac{\sum A_i \, MW_i}{\sum A_i} \qquad (2)$$

Where MW_i is the molecular weight of each identified fatty acid.

6 Processing of samples of extracted oil from sewage sludge

6.1 Biodiesel recovery and purification

The reacted extract (namely biofuel), having a total FAMEs content of about 30%, was distilled under vacuum (165-220°C, 2 mbar) to obtain a distillate (First Distillate, FD) having a FAMEs content of 88-92 wt.% (yield of biodiesel recovery of 95%, with respect to the amount of FAMEs initially present in the biofuel).

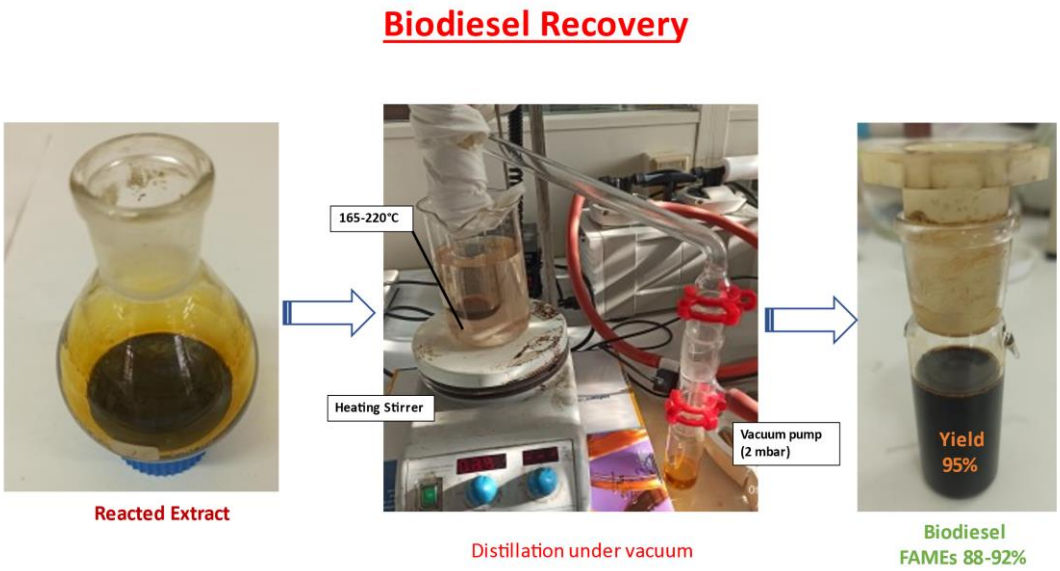


Figure 33: Biodiesel recovery through distillation under vacuum (T:165-220°C, p:2-4 mbar).

As a secondary product, a residue of distillation was also recovered, in which besides heavier FAMEs, other no polar compounds (heavy mineral oil, sterols, fatty alcohols, etc.) were dissolved in.

To improve the FAMEs content, a second distillation of FD was also performed. In this second distillation, a narrow temperature range was considered (165-180°C, 2 mbar), by collecting a purer fraction (Second Distillate, SD) having a FAMEs content of over 95%.

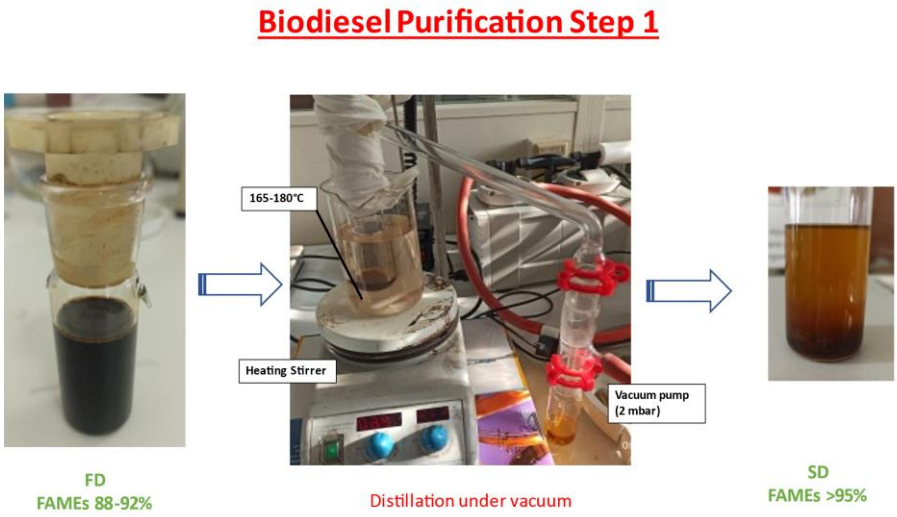


Figure 34: Biodiesel purification: second distillation under vacuum.

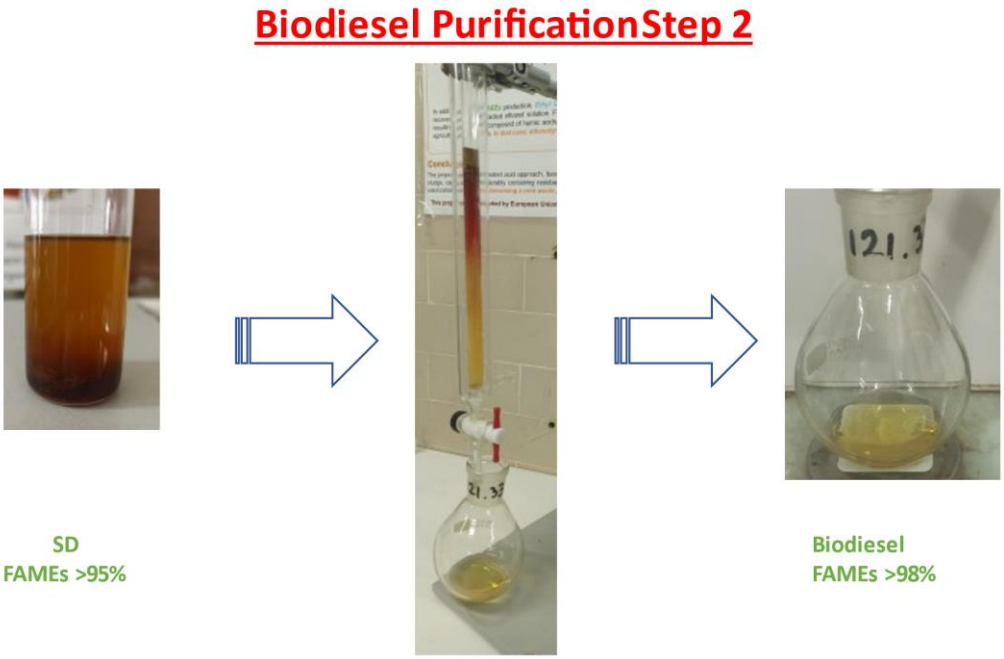


Figure 35: Biodiesel purification: use of SiO_2 as adsorbent.

Considering that the FAMEs content of 95 % makes the SD not respondent to the EN14214, for which at least a value of 96.6 % needed to be obtained, a further purification was necessary. A solvent-free washing was run using SiO_2 as adsorbent. At the end of this purification-step a very clean product was obtained (FAMEs title>99%).

Exhausted silica was also washed with methanol, allowing a fraction rich in fatty alcohol to be isolated.

With the aim of simplifying the purification step, the FD sample was also directly purified with the solvent-less adsorption on silica (First Distillate Cleaned, FDC).

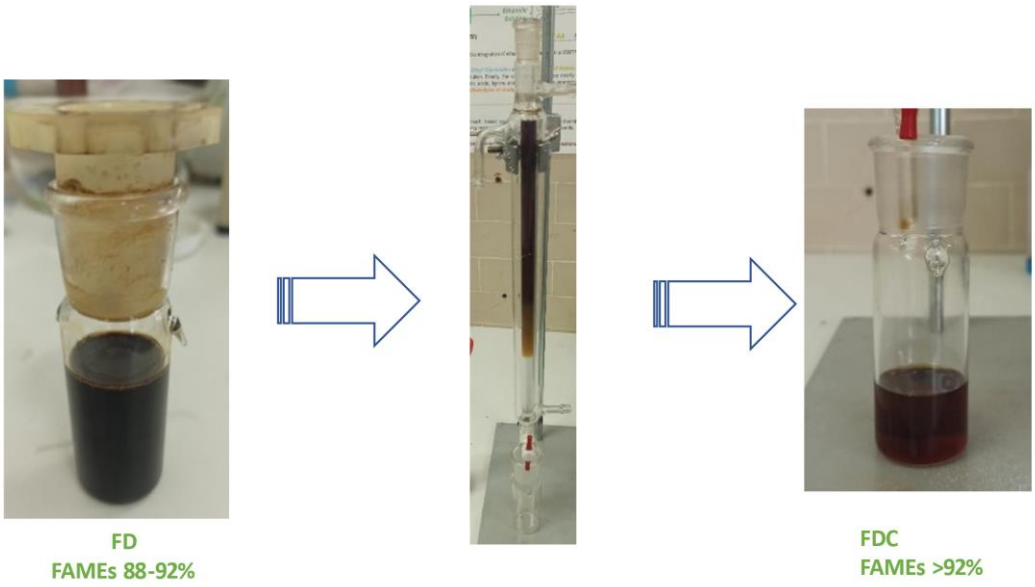


Figure 36: Direct solvent-less washing of FD using SiO_2.

Figure 37 shows the gas-chromatographic profiles of different samples, whereas Table 16 shows the results of the analysis related to SD, SDC and FDC, which can be considered the main samples of biodiesel obtained on this study.

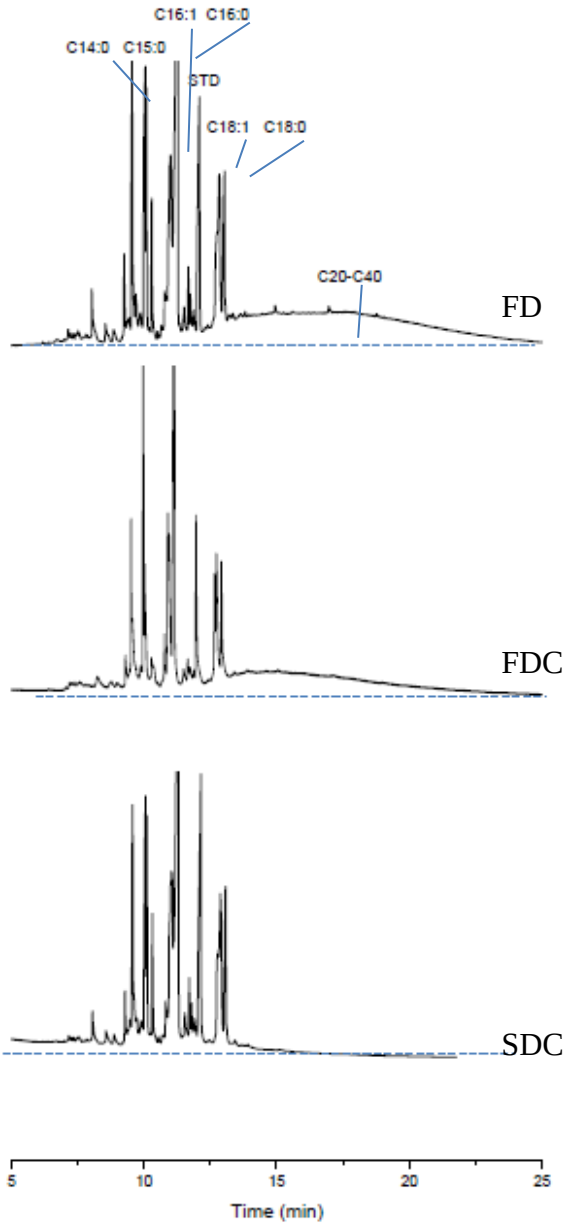


Figure 37: Gas-chromatographic profile of FD, FDC and SDC.

Table 16: Results of analysis carried out on biodiesel obtained after the second distillation under vacuum (SD), after dry washing on silica and the first distillate after dry-washing on silica (FDC).

Properties *(Units)*	**Lower limit**	**Upper limit**	**SD**	**SDC**	**FDC**	**Method**
Methyl Ester content *(wt.%)*	96.5	-	95	98.5	<92	*EN 14103*
Sulphated ash content *(wt.%)*	-	0.02	-	-	-	*ISO 3987*
Water content *(mg/kg)*	-	500	20	10	10	*EN ISO 12937*
Acid value *(mg KOH/g)*	-	0.5	<0.1	<0.1	<0.1	*EN 14104*
Iodine value *(g I_2/100 g)*	-	120	38	38.5	28.2	*EN 14111*
Methyl dodecanoate *(%)*	-	-	2.1	2.2	1.7	*EN 14103*
Methyl myristate *(%)*	-	-	8.4	8.6	6.6	*EN 14103*
Methyl pentadecanoate *(%)*	-	-	16.2	16.6	12.7	*EN 14103*
Methyl palmitate *(%)*	-	-	32.1	32.8	25.1	*EN 14103*
Methyl palmitoleate *(%)*	-	-	14.2	14.5	11.1	*EN 14103*
Methyl oleate *(%)*	-	-	13.4	13.7	10.5	*EN 14103*
Methyl linoleate *(%)*	-	-	0.6	0.7	0.5	*EN 14103*
Methyl stearate *(%)*	-	-	5.8	5.9	4.5	*EN 14103*
Linolenic acid methyl ester *(%)*	-	12	absent	absent	absent	*EN 14103*
Methanol content *(wt.%)*	-	0.2	<0.01	<0.01	<0.01	*EN 14110*
Monoglyceride content *(wt.%)*	-	0.7	absent	absent	absent	*EN 14105*
Diglyceride content *(wt.%)*	-	0.2	absent	absent	absent	*EN 14105*
Triglyceride content *(wt.%)*	-	0.2	absent	absent	absent	*EN 14105*
Free glycerine *(wt.%)*	-	0.02	absent	absent	absent	*EN 14105*
Total glycerine *(wt.%)*	-	0.25	-	-	-	*EN 14105*
Group I metals (Na + K) *(ppm)*	-	5	31.4	4.1	10.3	*EN 14108*
Group II metals (Ca + Mg) *(ppm)*	-	5	402	3.3	9.4	*EN 14538*

Figure 37 shows that after the first distillation there is a clear presence of C20-C40 hydrocarbons which were distilled in the range 190-220°C at 2 mbar. This product did not respect the minimum limit of FAMEs content considered for commercial purposes according to the EN14214 standards. However, such an aspect could be not so important for an applicative point of view, especially considering that biodiesel is often used in formulation with hydrocarbons. On the contrary, the high content of alkaline metals makes FD and SD not suitable for commercial purposes for the high level of alkaline metals. The second distillation under vacuum allowed a refined product to be obtained, unless the slightly low FAMEs content and the high content of metals of Group I and II with respect to EN14214 standard. **However, the dry washing of SD with silica, allow a biodiesel conform to the EN14214 to be obtained.** Considering that the dry washing with silica reduced the final metal content, FD was also directly treated with the aim of removing these salts. The preliminary results were promising and further improvable by increasing the Silica/biodiesel ratio. This strategy may become a valid alternative to produce a new biofuel of high quality without using a double distillation under vacuum.

6.2 Conclusions of processing the samples of extracted oil and production of biodiesel

Biofuel obtained from sewage sludge was used as a source of biodiesel. Two different routes of operations were designed and tested based on the distillation under vacuum and dry washing procedures with silica. When biofuel was distilled two times and treated with silica, a biodiesel (SDC) responding to the EN14214 standard was achieved. On the other side, already the first distillation under vacuum, coupled with the silica treatment, allow a new semi-refined product (FDC) to be isolated.

Processing 1 kg of desiccated foam, 65 g of biofuel were eventually recovered using a pretreatment with H_2SO_4 (50/350/500 H_2SO_4/sludge/MeOH weight ratio) and a liquid/liquid extraction with hexane. The distillation of this biofuel allowed 23.2 g FD (with FAMEs content of 90.5%) and 20.75 g SD (with FAMEs content of 95%) to be obtained respectively. From the purification on silica, 21.9 g and 19.35 g of FDC and SDC were eventually obtained.

In the present study, the main focus was the obtainment of biodiesel, but actually several other streams can be better valorised and necessitate further investigations. For example, a very consistent part of the initial biofuel (over 65% by weight) residue as an organic stream after the first distillation under vacuum. In the worst case, this residue of distillation can be considered as a biofuel. The correct estimation of costs/benefit ratio related to this train of technologies cannot be conducted by ignoring the fate of this stream.

6.3 Experimental Section 2

Sodium hydroxide (NaOH, 99%), potassium hydroxide (KOH, 85%), hydrochloric acid (HCl, 37%), sulfuric acid (H_2SO_4, 98%), diethyl ether ($(C_2H_5)_2O$, 99.5%), hexane (C_6H_{14}, 95%), methyl heptadecanoate (>99%), methanol (CH_3OH, 99.8%) and ethanol (C_2H_5OH, 99.8%) were purchased from Carlo Erba. Chemical reagents were of analytical grade and used without any purifications or treatments.

All the gas-chromatographic experiments were conducted in triplicate, allowing the average value and the standard deviations to be obtained. The mean value for each parameter was eventually reported, with a relevant variability that did not exceed the 5%.

Instruments

FT-IR spectra were collected using a Nicolet Summit FT-IR Spectrometer with an ATR accessory equipped with a diamond laminate crystal. Spectra were collected from 4000 to 400 cm^{-1}, with 64 scans per spectrum and 4 cm^{-1} resolution.

Identification of FAMEs was carried out by using a Perkin Elmer Clarus 500 gas-chromatograph interfaced with a Clarus 500 spectrometer. Quantitative determinations were performed with an Agilent 8890B GC system with flame ionization (FID) and thermal conductivity (TCD) detectors. Both instruments were configured for cold on-

column injections with an HP-5MS capillary column (30 m; Ø 0.32 mm; 0.25 µm film). The injector and the oven followed the same temperature program: an initial temperature of 40 °C was kept constant for 2.5 min, then was raised to 280 °C by using a 10 °C min^{-1} ramp and finally to 300 °C with 20 °C min^{-1}. The final temperature was kept constant for 15 min. Metal analysis (Na, K, Mg, Ca, Al,…) were carried out using a 7000X ICP-MS instrument (Agilent Technologies). 10 g of sample were calcined at 550°C for 3 h, then 10 mL of HNO_3 (67 %) were used to solubilize the residue under heating. The final solution samples were solubilized into 100 mL of Milli-Q water (0.6 $\mu S^2\ m^{-2}\ \Omega$), filtered, diluted and analyzed.

Gas-chromatographic determination of total content of FAMEs in final biodiesel

About 30 mg of sample were weighted in a vial together with 1 ml of methyl heptadecanoate standard solution (2 mg/ml in hexane). Then, 1 µL of the supernatant was injected into the gas-chromatograph. The total content of FAMEs was determined according to the Eq. 3

$$Total\ FAMEs\ (\%) = \frac{\sum A_i}{A_S} \cdot \frac{w_S}{w_{Biodiesel}} \quad 100 \qquad (3)$$

Where A_i is the area of the i-th fatty acid methyl ester detected by gas-chromatography, A_S and w_S are area and weight (mg) of the internal standard methyl heptadecanoate, respectively, and $w_{Biodiesel}$ is the amount of starting biodiesel (mg). In addition, the average molecular weight (*AMW*) was calculated according to the following equation (Eq. 4):

$$AMW = \frac{\sum A_i\, MW_i}{\sum A_i} \qquad (4)$$

Where MW_i is the molecular weight of each identified fatty acid.

Final Acidity of the biodiesel

Free fatty acids content were determined by dissolving the final biodiesel (2 g) in 50 mL of diethyl-ether:ethanol solution (1:1 v:v), and 0.1 mL of phenolphthalein indicator were placed in a 250 mL flask. Then, the resultant organic mixture was titrated with 0.1 N KOH solution until a phenolphthalein endpoint (pink coloration persisted for at least 30 s) was reached. The results were expressed as milligrams of KOH required to neutralize 1 g of the raw grease (mg KOH/g).

Distillation under vacuum

About 50 ml of biofuel or biodiesel were transferred and weighted into an appropriate equipment to perform the distillation under vacuum. A magnetic stirrer guaranteed the agitation during the evaporation. A thermostatic oil bath was used to heat the boiler of the distillation. Vacuum was generated by connecting the distillation

apparatus with an Edwards 3 vacuum rotaative pump up to 2-5 mbar. The temperature was gradually increased from 160 to 180/220°C, collecting FD and SD respectively.

In the first distillation, processing 65 g of biofuel (containing around 22 g of FAMEs), 23.2 g of FD were collected, whose FAMEs content was about 90.5%, for a final yield of recovery of FAMEs of 95.4 %. 41.5 g of residue of distillation were eventually recovered from the boiler (63.8% of the initial biofuel).

As for the second distillation, when 23 g FD (containing 41.6 g of FAMEs) were processed, 20.75 g SD (having a FAMEs content of 95%) was obtained, for a final yield of recovery of FAMEs of 94.7%.

Purification on silica

Purification on silica was operated using a column 20 cm long (inner diameter of 1 cm) containing about 5 g of Silica for the removal of polar compounds. In a typical experiment, 20.75 g of biodiesel (SD, 95% content of FAMEs) were directly eluted on that column by recovering the purified product under atmospheric pressure (SDC). To complete the recovery of the biodiesel, 5 ml of hexane were eluted. 19.35 g SDC were eventually collected (98.1 % of FAMEs), to achieve a final yield of 96.3%. 5 ml of methanol was eventually eluted to collect 0.5 g of polar compounds.