Determination of the ^{14}C content in Biodiesel: A method improving the detection sensitivity by decolorizing the biogenic material in biofuel.

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Revvity

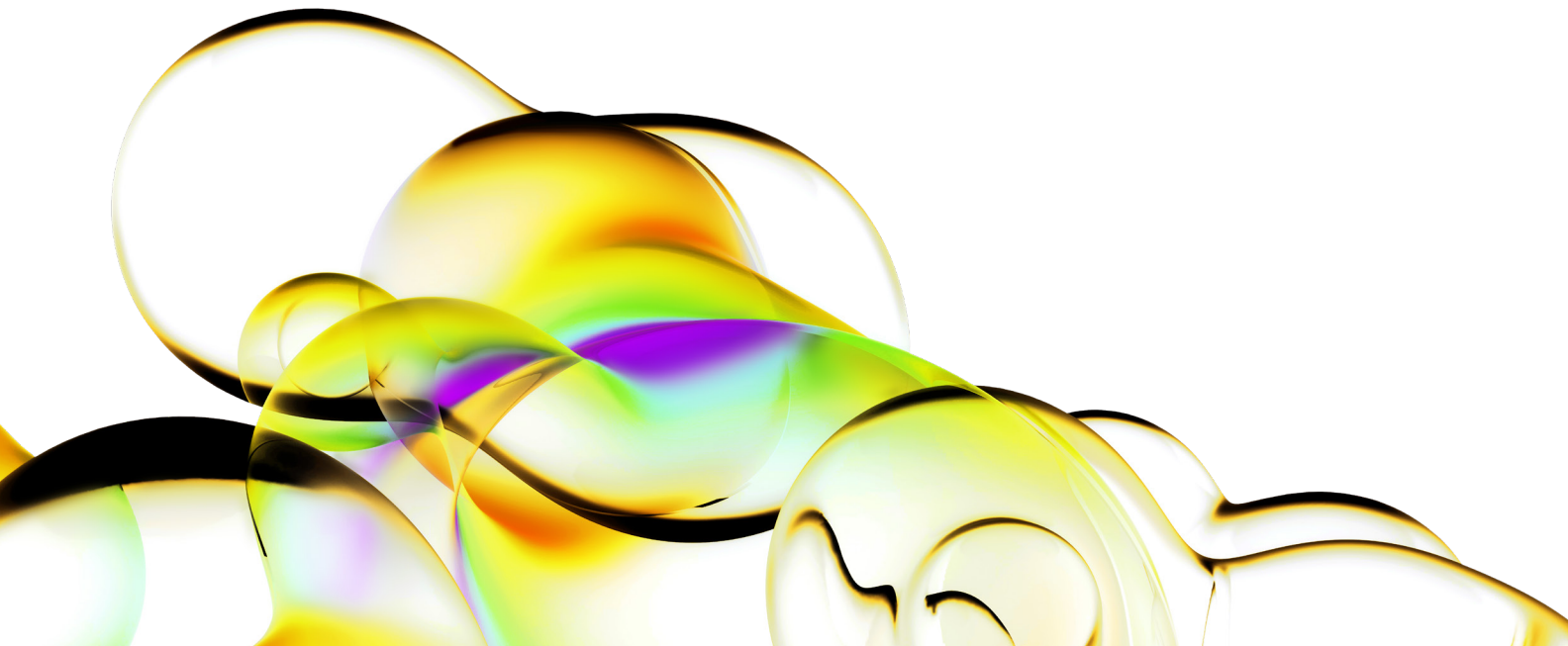
Introduction

There are at least two good reasons for the addition of biogenic materials to fuel. Of course, we have to be very careful with our limited fossil resources and secondly, we should decrease the emission of fossil CO_2 into the atmosphere.

The so called Kyoto protocol is related to the reduction of gas emissions which contribute to the global increase of temperature and produce the „greenhouse" effect. The reduction of gas emissions can be achieved by alternative energy sources such as wind energy, solar energy etc. but also by addition of biogenic materials to fuel. The addition of biogenic materials is regulated in directive 2003/30/EC and dictates the addition of at least 5% biogenic material to fuel. The additives can originate from the addition of biogenic ethanol or can be produced from biogenic ethanol. Another important source of biogenic materials is biodiesel originating from fats and oils. Several oils are transferred to biodiesel by transesterification processes making the natural oil suitable for applications in diesel engines.

A method to quantify the amount of biogenic material in the fuel is using liquid scintillation counting based on the presence of the ^{14}C in materials of natural origin. Recently some papers reported about LSC for the detection of ^{14}C in biogenic materials in fuel.[1-11)]

Most of these articles are related to the quantification of colorless bioethanol which makes it very simple to determine ^{14}C with LSC due to the absence of color quench.

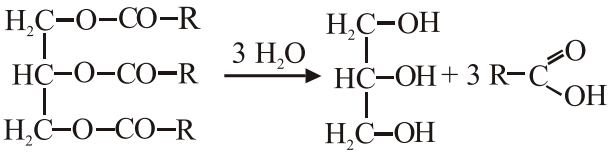


On the other hand, biodiesel in many cases has a rather intense color and the quantification by LSC is disturbed by very often yellow-colored components in the biodiesel. Strong color quench can reduce the counting efficiency significantly which also reduces the sensitivity of the LSC technique, and a correction can be complicated. In this application note we describe a method to remove the color from biodiesel and provide a method to increase the sensitivity of the LSC measurement to improve the detection of ^{14}C in biodiesel.

Materials

A 250 ml sample biodiesel, produced on the 10th April 2010, was obtained from Biovalue in Eemshaven, The Netherlands. This factory is producing biodiesel from rapeseed (Brassica Napus) originating from all over the world. This sample was rather yellow in color which is typical for biodiesel samples that originate from natural sources.

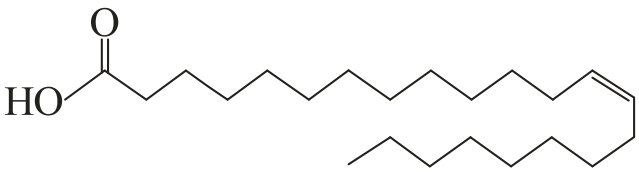
The process to prepare biodiesel is using a transesterification procedure to convert the triglycerides to the fatty acid methyl esters catalyzed by potassium hydroxide. As a side product glycerol and potassium sulfate are obtained.



Triglyceride ⟶ Glycerol + Fatty Acid

fatty acid + methanol ⟶ FAME

Erucic acid is the main component in rapeseed oil:



A small sample of biofuel from a customer (Fig. 1) containing a ready to use fuel also showed an intense yellow color.

Figure 1: Biodiesel in 6 ml glass vial

Ultima Gold F (Revvity part. no. 6013179) was used as LSC cocktail, counting was performed in 20 ml polyethylene vials (part no. 6008117) or 6 ml mini vials (part no. 6000292 or 6000167). For more details about cocktails please also read application note.[12)]

Counting was performed on a Tri-Carb™ 2550TR/AB in normal count mode at a temperature of 16°C. Unless otherwise specified, ^{14}C counting was done in an energy window from 0 – 156 keV. The older Tri-Carb model 2550 is comparable with the Tri-Carb 3110TR with Low Level Count Mode and α/β-discrimination of the current Tri-Carb series.

All the measurements obtained from a Quantulus™ were performed at an operating temperature of 18°C. Counting was performed in several cycles with a counting time of 60 minutes for every sample. Hexane p. a. was purchased from Acros.

The purification of the biogenic samples was performed at room temperature in glass chromatography columns. The following column materials have been used for the purification:

- Aluminum oxide 90 Merck, basic (0.063 – 0.200 mm)
- Silica Gel Merck, Silicagel 60 (0.040 – 0.063mm)

Biodiesel samples in most cases show intense yellow color. We analyzed samples with strong yellow color but depending on the origin of the sample the degree of color can vary between strong yellow and light brown. These colors could also be seen in samples that we investigated.

Some efforts have been reported (LSC 2010 Paris) to decolorize biofuel and biodiesel components but colorless or slightly colored material was not obtained. In this application note we report in some examples about the decolorization of biodiesel samples.

Decolorization of Biodiesel

The investigated fuel contained biodiesel and even at a low concentration of about 5% the colour was still strong yellow. The composition of this material was unknown. For counting an almost colourless fuel was available; this sample contained an unknown amount of bio derived components.

In order to obtain some information about quench levels we used colorless laboratory grade heptane.

The purification was done on a small glass column with 1 cm of diameter, filled with both a small amount of aluminum oxide and silica. 4 cm of the column height was filled with both materials. The silica material was on top of the column. The fuel material was given on top of the dry column and the fuel was allowed to run through the column by gravity. The fuel runs relatively fast through the column because it is not viscous at all. A small fraction of about 3 ml almost colorless material was obtained. The yellow component in the fuel was absorbed by the Silica on top of the column. Fig. 2 shows a part of the column with the yellow components of the fuel.

Figure 2: Glass column filled with aluminum oxide at the bottom and Silica on top of the column

The colored components are enriched in the upper part of the column after the separation procedure. A similar result is obtained when Biodiesel solutions in heptane are purified with the help of a column treatment.

Counting of the samples was done in a Tri-Carb 2550TR/AB at 16°C (CPM in an energy window 0 – 156 keV). We added 3 ml of sample in glass vials (Revvity part. no. 6000167) to 3 ml Ultima Gold F cocktail. The data of these measurements are shown in table one and two. The "Fuel" mentioned in the table was a colorless sample; the biodiesel sample was yellow colored.

Table 1: Measurement of colored and uncolored fuel

Cycle1	CPM	tSIE	% Eff.	% Lum.
Heptane	14.3	742	96.1	1
Fuel	36.1	746	96.1	1
Biodiesel (yellow)	15.2	414	92.1	11
purified	14.1	680	95.9	1

Table 2: Measurement of colored and uncolored fuel

Cycle2	CPM	tSIE	% Eff.	% Lum.
Heptane	14.5	746	96.1	1
Fuel	37.1	741	96.1	0
Biodiesel (yellow)	14.6	413	92.1	1
purified	13.4	678	96.0	0

Result:

Table one and two clearly show the high tSIE-values for almost unquenched heptane and colorless fuel. In contrast the tSIE-value and the efficiency in biodiesel decreased significantly. We also see that the tSIE-values of purified biodiesel are close again to the values of colorless reference samples. The counting efficiency again is close to the values of the colorless samples. All colored components remained on the column. We also noticed that initially observed luminescence was not present anymore in the material after the column purification. The ^{14}C counting efficiency increased from 92 to 96% after the purification.

Decolorization of a heptane solution of biodiesel

Biodiesel is readily soluble in heptane as well as in other organic solvents including LSC „safer" cocktails for lipophilic samples such as Ultima Gold F. In order to test the column purification methodology, we prepared a 10% solution of biodiesel in heptane and gave this solution on top of the dry column as described above. The column contents again consisted of a silica layer on top and an aluminum layer at the bottom. Approximately 30 ml of a 10% heptane solution was processed with this relatively small column. Fractions of 3 ml biodiesel solution have been collected.

Table 3: Data of fractions from heptane solutions after purification

	CPM	tSIE	% Eff.	% Lum.
Original	16.1	438	92.7	1
Fraction 1	13.6	725	96.1	1
Fraction 2	15.4	690	95.9	1
Fraction 3	16.7	699	96.1	1
Fraction 4	16.3	700	96.1	1
Fraction 5	16.3	677	95.8	1
Fraction 6	16.4	713	96.1	1

Initially a completely colorless fraction was collected while the next and following fractions contained some light yellow/green color.

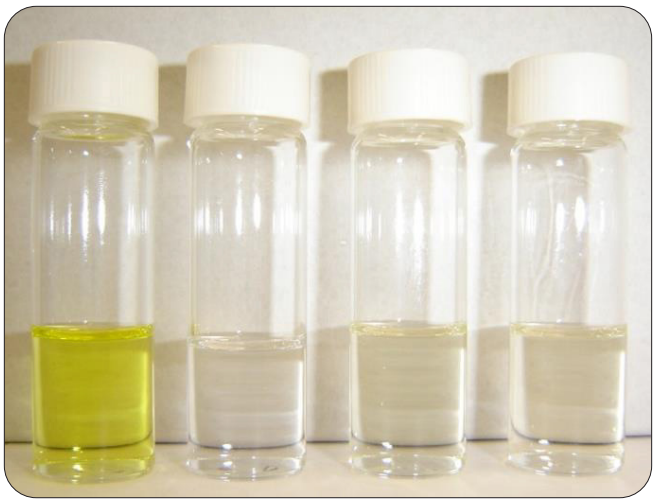


Figure 3: Decolorized samples of biodiesel dissolved in heptane

The left most vial is the original 10% biodiesel in heptane and the next fraction contains colorless sample eluting first. The two vials on the right side contain the material which still contains a very small amount of colored compounds. We added 3 ml of Ultima Gold F to these fractions into glass vials and counted in a Tri-Carb 2550 TR/AB. The counting time was 120 minutes and CPM-values have been obtained in an energy window from 0 – 156 keV. A total of six consecutive collected fractions were counted.

Result:

The ^{14}C efficiency increased by 3.5 to 4% compared to the untreated starting material. The fractions one and partly two have a lower count rate compared to the other vials counted. This is a result of the much higher (or exclusive) amount of heptane in these fractions eluting from the column in the beginning of the cleaning process. Heptane is almost eluting in the solvent front while other components move slightly slower through the column. The quench parameter is also an indicator of the improved counting efficiency after the purification. The CPM-values obtained except for the first vial are all close to 16.5 CPM and comparable with the 16.1 CPM in the original sample. In our opinion the removal of the yellow color helps to increase the ^{14}C counting efficiency. It is also important to note that the count rate remains stable after the treatment and is equivalent to the CPM in the original sample of 10% biodiesel in heptane. This means that we can exclude that much biogenic material remains on the column which could result in too low activities in our samples.

Decolorization of pure 100% biodiesel samples

The yellow biodiesel sample can be purified by using a combination of treatments. In general, the 100% biodiesel was heated up and stirred with some color absorbing material and then the warm mixture was given on top of a chromatography column. Initially the sample is running fast through the column but while the mixture is cooling in time the sample is running slower. At the end an almost colorless biodiesel can be obtained.

50 g biodiesel was treated with a few g of aluminum oxide (basic) and a few hundred mg of active carbon and stirred two hours at 50°C. Filtration of this sample is a difficult task. A small pore size filter is necessary to get rid of all the carbon but on the other hand this type of filter does not work with the high viscosity of biodiesel.

It works much better, when the still warm solution is directly given on top of the chromatography column, again filled with aluminum oxide and silica. An almost colourless eluate can be obtained.

Set up of the purification:

1. A sample of 50 g biodiesel was stirred with a few g of aluminum oxide and charcoal two hours at 50°C.
2. A column was prepared in a glass column with a diameter of 2 cm. The column was filled in the following ways:
3. Two different methodologies were followed:
    a. Test 1: The column was filled with 10 g of aluminum oxide resulting in a height in the column of approximately 4 cm.
    b. Test 2: The column was filled with 10 g of aluminum oxide and 2.5 g of silica resulting in a height in the column of 7 cm.
4. Next, the warm mixture of biodiesel and carbon was given on top of the column and the material was allowed to flow through the column by gravity.
5. Fractions of approximately 2.5 ml were collected for further counting in mini glass vials.

The result of the purification is shown below in Figure 4. The left most vial in the picture contains heptane, followed by original biodiesel. The other three vials contain fractions eluted from the column. Most of the intense yellow color could be removed from the crude biodiesel and the resulting sample contained an only slightly yellow colored material and was free from strong quenching components.

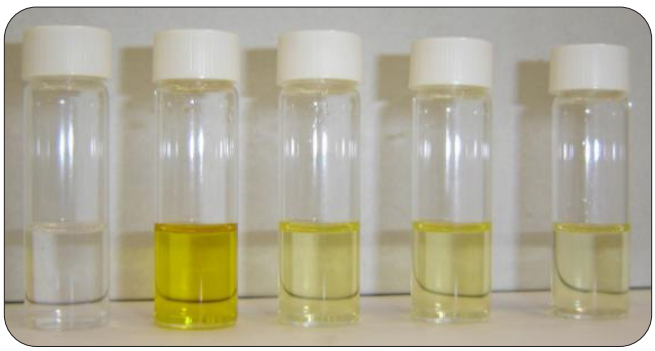


Figure 4: Decolorization of pure biodiesel

Quantification of the samples:

2.5 ml purified material was added to a plastic mini vial which was placed in a 20 ml plastic vial (Revvity part no. 6008118). 2.5 ml Ultima Gold F was added, and counting was performed in the open energy window from 0 -156 keV with a counting time of 120 minutes. The data are summarized in table 4 for the purified biodiesel; table five is summarizing the counting efficiencies of the samples. The heptane solutions were used as an indication of background and quench levels in uncolored samples.

For the calculation of theoretical DPM values we used a density of 0.86 g/ml for biodiesel and a modern carbon activity of 14 DPM/g carbon. We used the molecular formula of erucic acid as the model compound for biodiesel to calculate the theoretically expected DPM values. This of course is a simplification because the amount of erucic acid in the fatty acids of rapeseed oil usually does not exceed 42%.

Table 4: CPM values of biodiesel and purified samples

	CPM	tSIE	DPM	net DPM	Theory
Heptane	11.9	777	12.5	0	0
Original	20.2	94.7	27.1	20.4	23.5
Test 1,1	33.2	571	35.3	23.4	23.5
Test 1,2	32.5	557	34.5	22.6	23.5
Test 2,1	33.6	619	35.5	23.6	23.5
Test 2,2*	32.1	658	33.7	21.8	21.95

Test 2,2* is a sample with 2 g biodiesel.

The ^{14}C counting efficiency listed in table five was determined for the samples in table four by adding a ^{14}C internal standard to each individual vial (8972 DPM/vial). The measurement was done in an energy window from 0 – 156 keV. The DPM-values of the samples were obtained by using Ultima Gold quench curves set up on the Tri-Carb instrument. The obtained DPM-values are in good correlation with the amount of ^{14}C label added (variation ± 1 %) to the individual vials.

Table 5: Efficiency of biodiesel samples

	CPM	tSIE	DPM	% Eff.
Heptane	8570	785	8970	95.5
Original	3657	94	4901	40.8
Test 1,1	8464	572	8990	94.3
Test 1,2	8480	566	9012	94.5
Test 2,1	8435	622	8923	94.1
Test 2,2*	8599	685	9064	95.8

One exception can clearly be seen in table five. The DPM-value of untreated biodiesel does not show the expected value. This is a result of the extremely high quench level in this sample, which could not be handled by the used quench curve. The software had to use extrapolation algorithms which lead to an erroneous result in this case. Also the ^{14}C quench curve can only correct for chemical quench in colorless samples.

However, the correction of color quench results in an increasing error with increasing color quench.

Table four also shows a deviation for the untreated biodiesel sample for CPM (DPM) values compared with the expected values. The extremely high quench level results in a bit lower DPM-values than expected.

Measurements with the Quantulus:

Comparable measurements have also been done with the Quantulus. 5 ml Ultima Gold F were given into Teflon Vials (Revvity part no. 1220-500). We prepared 4 samples:

1) Blank: Cocktail with 1 ml heptane

2) 1 g biodiesel untreated

3) 1 g purified biodiesel

4) 100 mg biodiesel untreated

Table 6: Biodiesel measured in the Quantulus

	SQP	CPM Channel 50-650	net CPM channel 50-650	Eff%	mg sample	DPM netto	DPM expected
blank	839	0.597		75.7		0	
1 g biodiesel	490			0.365 #	1000		
1 g purified	829	8.657	8.060	75.5	1000	10.69	10.97
100 mg biodiesel	713	1.235	0.638	62.6	100	1.180	1.097

From table six we can clearly see a problem. An untreated biodiesel sample results in very low count rates and the counting efficiency drops to very low levels. The count rate is even lower than the count rate of the background sample (heptane) therefore background corrected results cannot be determined in the 1 g sample of pure biodiesel.

For the purified samples on the other hand the background correction results in reasonable data but here the quench level of the purified sample and the background sample are in the same range.

The measurements seem to confirm that it is difficult to obtain reliable data from untreated biodiesel samples. It is known that quench can also have an influence on the background count rate and obviously this has to be taken into account when measuring untreated biodiesel samples with strong color quench.

Measurements of small biodiesel samples with the Quantulus:

We prepared a series of untreated biodiesel samples and measured these samples in the Quantulus. All biodiesel samples were measured in 10 ml Ultima Gold F in a 20 ml plastic vial. The amount of biodiesel ranged from 52.9 to 302 mg of sample. The samples were kept at least two hours in the dark before we started counting in the Quantulus in 10 cycles. The measurement time was 60 minutes for every individual sample. The data are shown in table 7.

Table 7: Measurement of different amounts of biodiesel in Ultima Gold F in the Quantulus.

Sample	mg biodiesel	SQP	50 - 650	50 - 700	CPM - blank	^{14}C Eff. %	dpm	dpm/g
1	0	938	1.054	1.14				
2	0	939	0.974	1.061				
3	52.9	913	1.518	1.602	0.504	87.1	0.578	10.9
4	93.5	891	1.924	2.047	0.910	85.2	1.068	11.1
5	161.6	861	2.446	2.55	1.432	83.3	1.718	10.6
6	203.5	847	2.857	2.985	1.843	80.6	2.286	11.2
7	242.9	834	3.135	3.234	2.121	79.1	2.681	11.0
8	302.4	820	3.599	3.69	2.585	77.7	3.326	11.0

We obtained the data in table 7 from 6 samples containing different amounts of biodiesel in 10 ml Ultima Gold F as well as from two background samples in plastic vials. The amount of biodiesel was weighed as mentioned in the table. After the measurement we calculated the background corrected CPM values via subtraction of the average background of the two background samples. The 14C counting efficiency was obtained from a quench curve especially made in Ultima Gold F (details will be given below) using specified quantities of a yellow dye in Ultima Gold F. For further details about quench curves please also read the LSC application notes referenced in the literature section.[13, 14] From the quench curve we calculated the DPMvalues and the DPM/g biodiesel.

Although the data for every measurement point only rely on a single measurement, the value for DPM/g biodiesel is very constant as one would expect from theory. In other words, we can see a linear relation between the activity of the sample and the measured DPM-values which becomes obvious in figure 5.

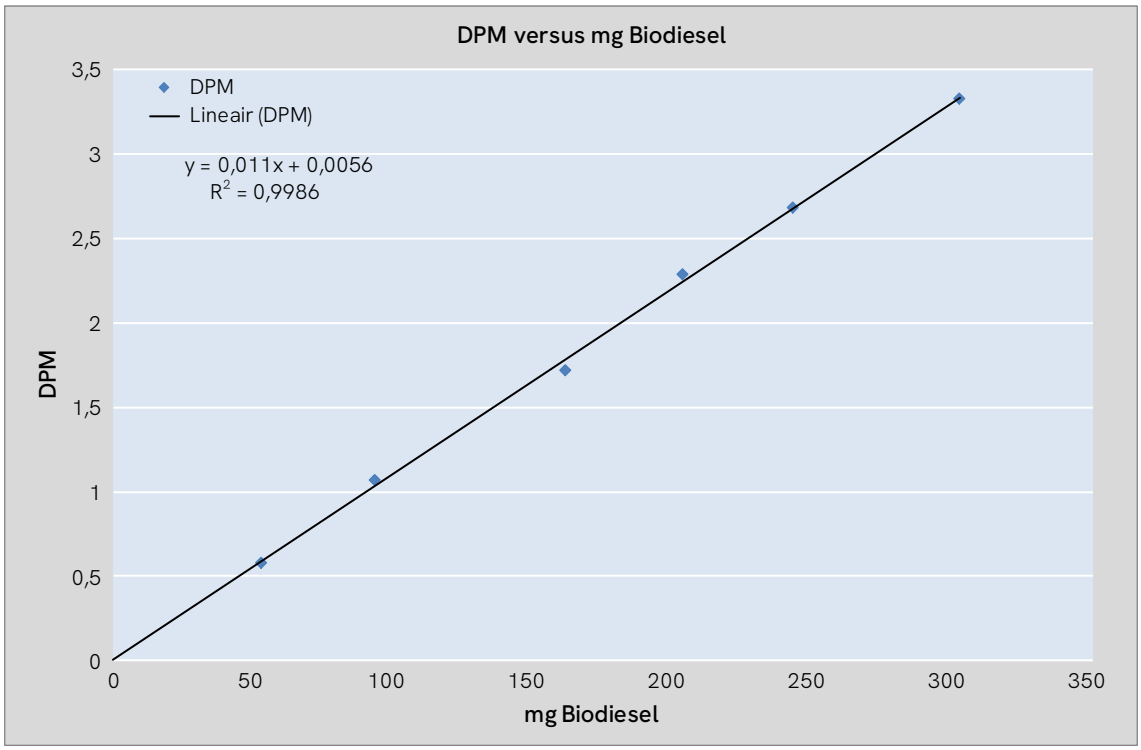


Figure 5: DPM-values versus amount of biodiesel measured in the Quantulus.

Preparation of quench curves for the Quantulus

The color quench in biodiesel samples is very much depending on the origin of the biogenic material. Biodiesel from rapeseed has a much stronger yellow color than biodiesel from sunflower oil.

Therefore, we have prepared a series of yellow-colored samples in Ultima Gold F with increasing amounts of a yellow dye. We used the yellow dye dimethyl azobenzene in a concentration of 20 mg/100 ml of Ultima Gold F. All vials contained the same amount of activity of a ^{14}C labeled material (9075 DPM). We selected samples from a series of 15 vials in a way that the DPM-activity was the same within a very small variation (14C DPM ± 1 %). To these vials we added a certain amount of the yellow dye solution. The vials have been counted on the Quantulus in five cycles with a counting time of 10 minutes for every sample in the energy range from channel 50 to 650 (0.84 – 160.7 keV). The data are summarized in table eight:

Table 8: Raw data of a quench curve prepared with yellow dye in Ultima Gold F.

Sample	µl yellow dye	SQP	CPM	^{14}C Eff.
1	0	940.0	8087.5	89.1
2	0	935.4	8027.3	88.5
3	50	864.7	7562.1	83.3
4	100	821.3	7052.2	77.7
5	150	779.0	6464.3	71.2
6	200	747.9	5769.2	63.6
7	300	711.6	4807.1	53.0
8	500	672.8	3403.4	37.5
9	700	641.0	2293.8	25.3
10	1000	603.9	1353.5	14.9
11	1500	574.5	788.4	8.7
12	2000	560.9	457.6	5.0
13	2500	535.8	303.2	3.3

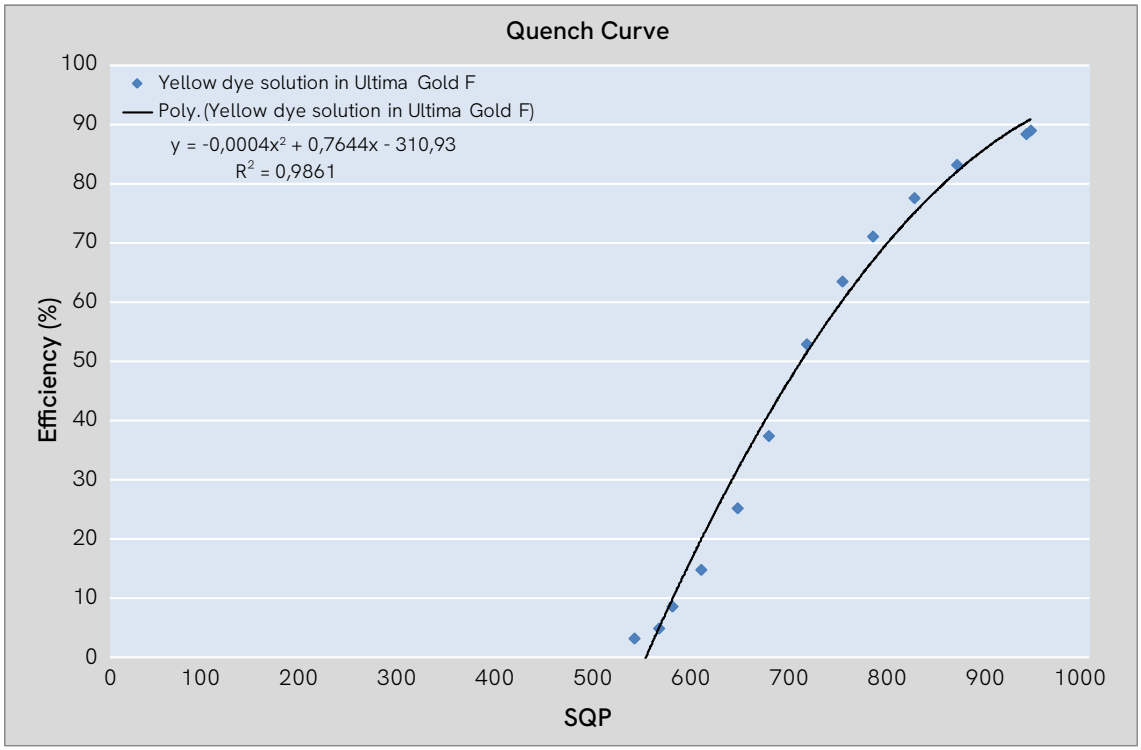


Figure 6: Quench curve with yellow dye in Ultima Gold F prepared with the Quantulus.

Measurements of small biodiesel samples with the Tri-Carb

Again, a series of biodiesel samples was measured on the Tri-Carb 2550. The samples were dissolved in Ultima Gold F (10 ml) and measured in plastic vials (20 ml). The quantities of biodiesel ranged from 47.2 to 2043 mg per vial. Samples were prepared by weighing the biodiesel into the vials.

After stabilization of the samples in the dark at 16°C for at least two hours counting was performed for 180 minutes for every sample in the Tri-Carb 2550 in the energy window from 0 – 156 keV. The DPM of the sample was determined using an Ultima Gold quench curve and the tSIE as quench parameter. Table nine is presenting the CPM- and DPM-values as determined by the instrument. The blank corrected values have been obtained after subtraction of the average of two background samples.

Table nine also contains the activity per g biodiesel which has been calculated from the weighed amount of biodiesel.

The CPM, DPM and DPM/g data in table 9 indicate the presence of a large linear range. The DPM/g value is between 10 and 11 DPM. Only the first two samples deviate from these values because they are close to the detection limit of the Tri-Carb 2550. Deviations can also be seen at very high amounts of biodiesel. In case the amount of biodiesel is higher than 1.5 g per vial the DPM/g value decreases significantly. This is due to the increased amount of color quench which cannot be corrected by a quench curve for chemical quench alone. It is known that chemical quench and color quench samples especially at strong quench levels show different energy distributions even at identical tSIE-values. This is the reason why strong color quench requires a color quench curve with a yellow dye which comes much closer to the real conditions in a biodiesel sample. Nevertheless, also chemical quench exists in biodiesel samples making it even more complicated.

Table 9: Measurement of biodiesel in the Tri-Carb 2550 with Ultima Gold F

Sample	mg biodiesel	tSIE	CPM	CPM - blank	DPM	DPM - blank	DPM/g
1	0	1109	15.6		16.3		
2	0	1110	15.9		16.7		
Average	0		15.8	0.0	16.5	0.0	
3	47.2	916	16.2	0.4	16.9	0.4	7.8
4	95.3	813	17.7	1.9	18.4	1.9	19.5
5	196.5	605	16.7	0.9	18.5	2.0	10.1
6	297.5	483	18.7	3.0	19.9	3.4	11.3
7	403.3	387	19.6	3.9	21.0	4.5	11.3
8	526.4	334	20.1	4.3	21.8	5.3	10.1
9	708.2	273	21.7	5.9	23.9	7.4	10.4
10	1002.5	207	23.7	7.9	26.9	10.4	10.4
11	1502.7	155	25.9	10.1	30.9	14.4	9.6
12	2043.6	128	27.4	11.6	33.9	17.4	8.5

Color quench curves on the Tri-Carb 2550:

The factory installed quench curves for correction of chemical quench on the Tri-Carb are not fully comparable with the yellow sample material in biodiesel therefore we have made a curve based on the yellow dye. The samples again contained exactly 9075 DPM in each vial and were prepared in Ultima Gold cocktail. To these samples we added the amount of yellow dye mentioned in table 10.

All samples were counted on the Tri-Carb 2550 TR/AB in an open window from 0 – 156 keV with a counting time of 10 minutes at 16°C. The quench set prepared this way should cover the quench range of real biodiesel samples much better than a normal quench curve for chemical quench correction only.

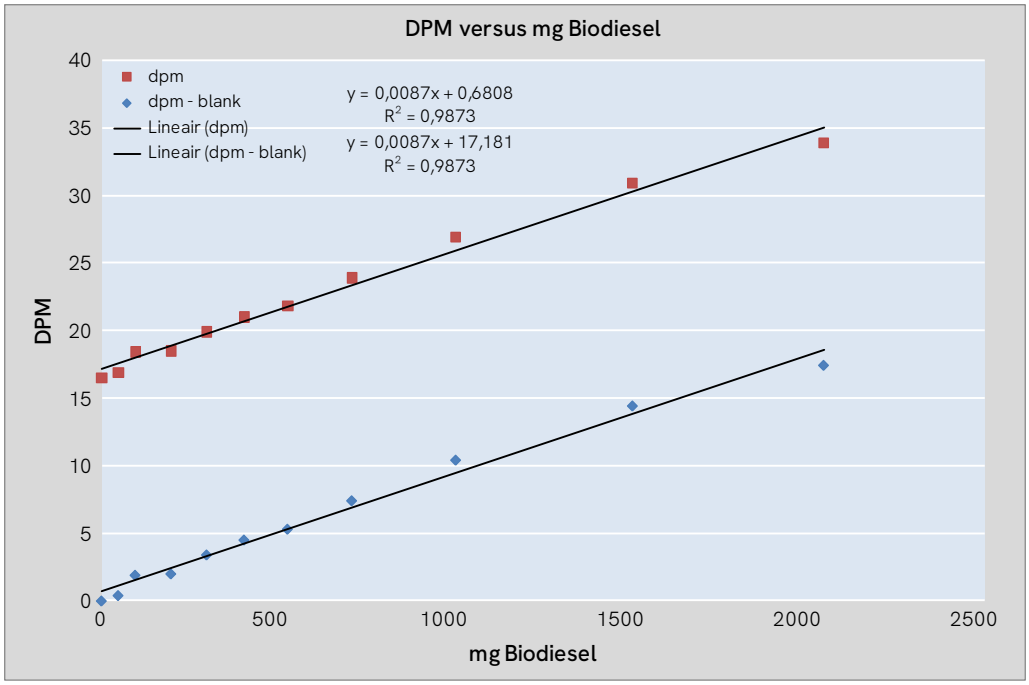


Figure 7: DPM-values and blank corrected DPM-values versus amount of biodiesel measured on a Tri-Carb 2550 TR/AB

Table 10 contains the data obtained from the quench set produced with the yellow dye and in contrast to this table 11 is presenting the data for the quench curve which is based on a colourless quench set for the correction of chemical quench only. As the above evaluations already indicated, measurements of colored samples done with a tSIE-value of more than 200 results in acceptable data even when using a colorless chemical quench curve. Stronger color quench requires the preparation of a quench set using an appropriate dye. Figure eight offers the data from table 10 and 11 in a graph.

Table 10: Color quench curve on the Tri-Carb

Probe	µl Farbstoff	tSIE	CPM	^{14}C Eff.
1	0	1107	8702	95.9
2	0	1106	8704	95.9
3	50	691	8583	94.6
4	100	487	8438	93.0
5	150	370	8242	90.8
6	200	295	7921	87.3
7	300	222	7591	83.6
8	500	166	6846	75.4
9	700	127	6342	69.9
10	1000	99	5490	60.5
11	1500	80	4644	51.2
12	2000	66	3827	42.2
13	2500	57	3274	36.1

Table 11: Chemical (colorless) quench curve on the Tri-Carb

^{14}C Eff.	tSIE
95.9	797
96.2	677
95.1	582
94.1	481
93.1	390
91.3	297
88.7	215
82.5	140
75.5	98
60.1	58

From figure eight it becomes clear that samples with strong color quench that have been corrected with a quench curve for the correction of chemical quench only result in too high efficiencies which consequently leads to too low DPM values. This is exactly what we saw in table eight with the decrease of DPM/g at high color quench levels.

Conclusion:

The decolorization significantly reduced the color quench, increased the sensitivity and improved the reliability of the biodiesel measurements. Using this cleaning procedure samples give reliable data even without the preparation of a color quench curve.

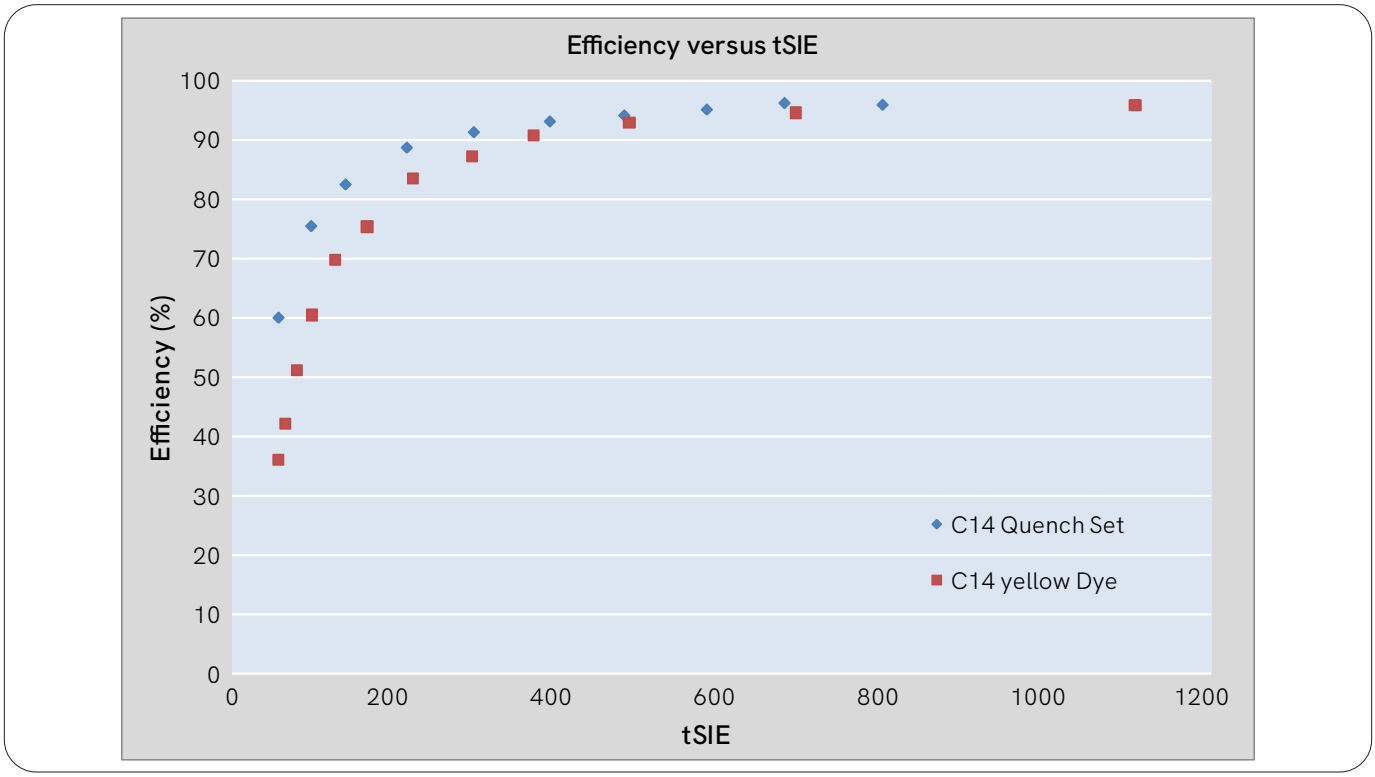


Figure 8: Comparison between a colorless quench set (blue) and a quench set with yellow dye (red)

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