07

Series "Biomass energy use"



ERRATUM Dec 2023

Collection of Methods for Biogas

Methods to determine parameters for analysis purposes and parameters that describe processes in the biogas sector

Biomass
energy use

Editorial notes

Editors

Tina Händler
DBFZ Deutsches Biomasseforschungszentrum
gemeinnützige GmbH
Torgauer Straße 116
D-04347 Leipzig
Tel.: +49 (0)341 2434-554
E-mail: tina.haendler@dbfz.de
www.energetische-biomassenutzung.de

Layout/Typesetting

Joshua Röbisch

Photos

Title: DBFZ, DBFZ, DBFZ, Susanne Beeck/pixelio

Funding

Created with financial assets of the Federal Ministry for Economic Affairs and Climate Action (BMWK)

ISBN 978-3-946629-47-4
ISSN (online): 2698-9190

Erratum DOI: 10.48480/f9ne-2j04

Erratum December 2023

Supported by:

Federal Ministry for Economic Affairs and Climate Action

on the basis of a decision by the German Bundestag

Project management

Projektträger Jülich Forschungszentrum Jülich

Service & support

DBFZ

Series "Biomass energy use"
Volume 7 // ERRATUM

Table of content

4.7 Determination of total Kjeldahl nitrogen and crude protein

Michael Goldstein, DBFZ

Status	The method is an in-house method that is carried out by modelling it after the prescribed method of VDLUFA, Book of Methods III, Testing of Feedstuffs, Determination of Crude Protein, Official Method, Hamburg 1988 (VDLUFA 1988).
Associated standards	Nitrogen determination according to Kjeldahl
Area of application of the method	Determination of the crude protein content of feedstuffs based on determined nitrogen contents (according to Kjeldahl)
Disadvantages	Lengthy. May be potentially falsified due to, for example, melamine (or other nitrogen sources) (non-specific method) The fixed factor must be adjusted, depending on the sample, where applicable.
Advantages	Nitro, nitroso and azo compounds are not being detected.
Need for research	For this method, there is no need for research.

Through an acidic thermal decomposition under catalyst involvement, protein(s) and other compounds containing nitrogen are split into ammonia. Ammonia is released by means of alkaline water vapour distillation and captured in boric acid. Subsequently, a quantitative determination of the ammonia takes place by means of sulphuric acid titration. The share of ammonia determined allows for conclusions regarding the nitrogen bound in the protein. For this, the factor 6.25 is used for the conversion of the nitrogen content to the crude protein content. The method is carried out according to the determination according to Kjeldahl.

Devices and chemicals

- devices: Turbosog, Turbotherm, Kjeldatherm, Vapodest 50sc
- decomposition vessels and accessories
- 1.5 L beaker
- 250 mL wide-neck Erlenmeyer flask
- drying cabinet
- precision scale
- cucible
- desiccator
- boric acid 2 %

- sulphuric acid (nitrogen-free) 0.025 mol L^{-1} (normality: 0.05 mol L^{-1})
- soda lye (nitrogen-free) 32 %
- ammonia sulphate $(NH_4)_2SO_4$ at least 99,5 % (p. a.)
- catalyst tablets ($CuSO_4 \times 5\ H_2O$, Na_2SO_4, Se)
- aqua dist.

Preparation of the analysis

The analysis is carried out on sample wet weight (fresh matter). Both liquid and solid samples can be analysed. At a minimum, a double determination is carried out for each sample.

Execution of the analysis

Thermal decomposition

For the analysis, approximately 0.3 g_{TS} (m_1) are weighed-in with an accuracy of 0.1 mg. Distilled water is used as blank reading, and ammonia sulphate [$(NH_4)_2SO_4$] as standard substance. Two catalyst tablets are placed in each decomposition vessel, covered with 20 mL 98 % sulphuric acid, and placed in the glass on a heating block. The suction extraction facility is placed on top of this. The samples are decomposed for 55 min at 230 °C and subsequently for 1:15 h at 390 °C, wherein the solution should have taken on a clear green colouring. Once the decomposition has ended, let it cool down for approx. 20 min. Subsequently, approx. 90 mL boiling water (aqua dist.) are layered underneath, running down the side of the glass.
To prevent the crystallising out of the sulphate, the samples are once again placed on the still warm heating block.

Alkaline water vapour distillation

The decomposition vessels are placed in the distillation device, and subsequent to addition of 66 mL soda lye, distilled for 5 min at 100 % steam output. Within the process, the distillate is transferred into 60 mL boric acid. Then the boric acid is titrated with 0.1 N sulphuric acid until pH = 5.

Calculation of the total Kjeldahl nitrogen content

$$TKN = \frac{(V_1 - V_0) \cdot c \cdot f \cdot 0{,}014}{m} \cdot 100 \qquad \textbf{08}$$

TKN	Total Kjeldahl nitrogen content	% FM
V_1	Volume of the sulphuric acid consumed when titrating the sample	mL
V_0	Volume of the sulphuric acid consumed when titrating the blank reading	mL
c	Normality of the acid	mol L^{-1}
f	Factor of the acid	
m	Mass of the sample	g

Calculation of the protein content

$$CP = TKN - \left(NH_4^+\text{-}N \cdot \left(\frac{100 - TS}{1000} \right) \right) \cdot 6.25 \quad \textbf{09}$$

CP	Protein content	% FM
TKN	Total Kjeldahl nitrogen content	% FM
NH_4^+-N	TAN (total ammonia nitrogen)	g L^{-1}
TS	Total solids content of the sample	%

For all samples, the dry matter must be determined in order to be able to put the result in relation to the total solids. In addition, the ammonia nitrogen content (TAN) must be measured in order to calculate the protein content.

4.9 Determination of crude fat

Michael Goldstein, DBFZ

Status	The method is an in-house method that is carried out modelled after the prescribed method of VDLUFA, Book of Methods III, Testing of Feedstuffs, Determination of Crude Fat, Ch. 5.1.1, Official Method, Procedure B, Hamburg 1988 (VDLUFA 1988).
Associated standards	Determination of crude fat, official method
Area of application of the method	Determination of crude fat in feedstuffs. Not suitable for oilseeds.

The sample is heated with hydrochloric acid in order to open up (decompose) proteins and release bound lipids. The decomposition solution is filtered and, after drying, the fat remaining in the filter is extracted with hexane. The solvent is distilled off and the dried residue is weighed. The fat content is calculated from the difference between the weighing-in and weighing-out.

Devices and chemicals

- soxtherm extraction unit Makro and Multistat device
- precision scale
- hydrolysis automaton "Hydrotherm"
- pleated filter with an average pore diameter of approx. 5 µm
- drying cabinet
- desiccator
- weighing paper, fat-free
- crucible
- pH indicator paper
- wadding, chemically pure and degreased
- extraction beaker(s)
- extraction sleeves
- sleeve holder(s)
- compressor at least 4.5 bar
- water supply at least 0.5 bar
- hydrochloric acid 3 mol L^{-1}
- hexane
- aqua dist.
- where applicable, liquid N_2
- where applicable, dry ice

Preparation of the analysis

Prior to the analysis, the fresh samples are ground to ≤ 1 mm; where applicable, they are embrittled for this by means of liquid nitrogen and solid CO_2 (dry ice). A double determination is carried out. The dry matter of the dried sample must be determined in order to be able to put the result in relation to the total solids.

Execution of the analysis

Hydrolysis

Approximately 2.5 g of a fresh sample – accurate to 0.1 mg – are placed on the weighing paper, which is then folded together. The paper, together with the sample, is put into a hydrolysis beaker to prevent baking onto the beaker's bottom while heating it up. Subsequent to the addition of 100 mL 3 mol L^{-1} hydrochloric acid, an automatic heating to boiling temperature takes place and is held for 1 h at mild simmering. It has proven advantageous to continue the simmering process until the complete decomposition of the substrate. Where applicable, rinse the border that occurred into the glass with some HCl and continue the simmering process. Subsequent to the completion of the hydrolysis, the decomposition mixture is drained into the prepared pleated filter and rinsed with hot distilled water. The pleated filters are rinsed 16 times with 40 mL distilled water, each. The filters should be pH-neutral (testing by means of Unitest paper). The filters are then placed on watch glasses and dried over night in the drying cabinet at 50 °C. Depending on the number of samples, the extraction beakers are dried with three boiling stones, each, for at least 1 h in the drying cabinet at 105 °C, or – preferably – over night at 50 °C.

Extraction

Subsequent to the cooling down in the desiccator, the extraction beakers are weighed accurate to 0.1 mg and the mass (a) is recorded. Subsequent to the cooling down in the desiccator, the dried filters are transferred into an extraction sleeve and covered with fat-free wadding. The prepared sleeve is placed in the appropriate holder and [then] placed into an extraction glass. Into this glass hold with round-nose pliers, 140 mL of fresh hexane are added. The glass is immediately placed in the ready-to-operate extraction unit. The extraction takes place according the programme described in Tab. 4.9–1.

After the completion of the programme, the extraction beaker is removed from the extraction unit and the extraction sleeves with the corresponding holders are removed and disposed of (and/or reused). The extraction beakers are dried in horizontal position for 2 h at 50 °C in the drying cabinet. After cooling down to room temperature in the desiccator, a weighing accurate to 0.1 mg is carried out and the mass (b) is recorded. Drying and weighing must take place immediately one after the other.

Table 4.9-1: Programme of the extraction unit

Programme step	Programme parameter(s)	Comment
T category	135 = < 200–300 °C	
Hot plate temperature	150 °C	
Lowering interval	4 min	
Lowering impulse	3 s	
Boiling phase	30 min	
Removal by distillation A	4 intervals	Subsequent to A the solvent level should be at least 10 mm below the sleeve
Extraction time	1 h	
Removal by distillation B	4 intervals	Subsequent to B the solvent level should be at least 10 mm below the sleeve
Removal by distillation C	2 min	

Calculation of the fat content

$$CF = \frac{b - a}{m} \cdot 100 \qquad (11)$$

CF	Crude fat content	%
a	Mass of the empty extraction vessel	g
b	Mass of the extraction vessel after the extraction	g
m	Mass of the dried and milled ample	g

4.10 Determination of crude fibre

Michael Goldstein, DBFZ

Status	The method is an in-house method that is carried out modelled after the prescribed method of VDLUFA, Book of Methods III, 2nd Supplement, Hamburg 1988 (VDLUFA 1988).
Associated standard	Determination of crude fibre, official method
Area of application of the method	This method determines the acid-insoluble and alkali-insoluble, fat-free, organic share in feedstuffs.
Disadvantages	Non-specific method, no indications regarding the individual fibre fractions.
Need for research	For this method, there is no need for research.

The dried sample is treated by boiling in H_2SO_4 and KOH. The undissolved residue is weighed out after drying and then turned to ash. The difference between the ash content and the undissolved residue is referred to as crude fibre. These skeletal substances essentially include: cellulose, hemicellulose, pentosans, lignin, cutin and pectin.

Devices and chemicals

- fibretherm FT 12 device
- fibrebag & accessories
- drying cabinet
- muffle furnace
- precision scale
- crucible & desiccator
- sulphuric acid 0.13 mol L^{-1}
- potash lye 0.23 mol L^{-1}
- hexane
- aqua dist.
- boiling stones

Preparation of the analysis

The samples must be dried in the drying cabinet at 105 °C for approx. 24 h and subsequently ground to ≤ 1 mm. Furthermore, for each sample a crucible must be calcinied empty at 500 °C for 2 h. A double determination is carried out. In addition, corresponding to the number of samples, Fibrebags must be dried in the drying cabinet at 105 °C for 1 h.

Execution of the analysis

Subsequent to the drying, the empty weight of the Fibrebags is determined. Then, approx. 1 g of dried sample must be weighed, accurate to 0.1 mg. A glass spacer is carefully inserted into the Fibrebags and together are placed in the sample carousel. All Fibrebags are thoroughly rinsed with a spray bottle filled with hexane. This way, excess fat is eluated from the samples. The sample carousel should be dried in the drying cabinet (105 °C) for approx. 5 min and be subsequently placed in the boiling container.

Table 4.10-1: Method for the determination of crude fibre

1	Dosage	H_2SO_4	1 L
2	Heating	45 %	0 h 30 min
3	Suctioning off		2 min/30 s
4	Washing cycle 1/2		
5	Washing cycle 2/2		
6	Dosage	KOH	1 L
7	Heating	40 %	0 h 30 min
8	Cooling	91 > 85 °C	
9	Suctioning off		2 min/30 s
10	Washing cycle 1/2		
11	Washing cycle 2/2		
12	Dosage	H_2O wash	1 L
13	Heating	50 %	0 h 5 min
14	Cooling	90 > 60 °C	
15	Method completed		

To determine the dried mass of the Fibrebags, first, the empty weight of an empty crucible calcined at 500 °C is determined. After removal of the spacer, the Fibrebag is placed in the crucible rolled up. The crucibles are dried for approx. 24 h at 105 °C, cooled down in the desiccator, and weighed. The ashing of the Fibrebags is carried out at 500 °C for at least 2 h. After cooling down, the samples are weighed. In addition, the dry matter of the analysis sample must be determined in order to be able to put the result in relation to the total solids.

Result calculation

$$CFC = \frac{(m_4 - m_1) - (m_5 - (m_6 - m_3))}{((m_2 - m_1) \cdot TS_{md})} \cdot 100 \cdot 100 \qquad (12)$$

CFC	Crude fibre content	$\%_{TS}$
m_1	Mass of the empty dried Fibrebag	g
m_2	Mass of the dried Fibrebag with sample	g
m_3	Mass of the empty crucible of the blank reading	g
m_4	Mass of the crucible & Fibrebag & sample after drying	g
m_5	Mass of the crucible & Fibrebag & sample after calcination	g
m_6	Mass of the crucible & Fibrebag after calcinationof the blank reading	g
TS_{md}	Total solids of the dried and milled sample	%

4.11 Process specification for the determination of ADF and ADL

Michael Goldstein, DBFZ

Status	The method is an in-house method that is carried out modelled after the prescribed method of VDLUFA, Book of Methods III, 2nd Supplement, Hamburg 1988 (VDLUFA 1988).
Associated standards	Determination of ADF and ADL, official method
Area of application of the method	This method determines the acid-insoluble components and the crude lignin of a sample.
Need for research	For this method, there is no need for research.

By boiling the dried samples in acidic ADF solution, cellulose, lignin and lignin-*N*-compounds are not eluated from the feedstuff. This undissolved residue is weighed out after drying. The residue remaining in the filter crucible in the determination of the ADF is treated at room temperature for 3 h with 72 % sulphuric acid. Subsequently it is rinsed with hot water to the neutral point, dried, and weighed. After ashing the organic substance, the substance is weighed again; the loss on ignition corresponds to the "crude lignin".

Devices and chemicals

- fibretherm FT 12 device, fibrebag (ADF) & accessories
- drying cabinet and Muffle furnace
- precision scale
- 5 L beaker
- crucible & desiccator
- acidic ADF solution
- hexane
- 72 % sulphuric acid
- aqua dist.

Preparation of the analysis

The samples must be dried in the drying cabinet at 105 °C for approx. 24 h. Furthermore, for each sample two crucibles must be calcinied empty at 500 °C for 2 h. The dried samples are ground with a mill to ≤ 1 mm. A double determination is carried out. The Fibrebags must be dried in the drying cabinet at 105 °C for 1 h.

Manufacturing of detergents for the ADF determination

Devices

- 5 L volumetric flask
- 50 L volumetric bulb pipette
- top unit scale
- glass funnel
- 250 L beaker
- piston pipette
- small weighing bowl(s)

Chemicals

- aqua dist.
- sulphuric acid (H_2SO_4) 98 %
- *N*-cetyl-*N*,*N*,*N*-trimethyl ammonium bromide

Manufacturing of the ADF solution

In a 5 L volumetric flask, approx. 2 L distilled water are placed and 136 mL concentrated sulphuric acid are pipetted in. In addition, 100 g *N*-cetyl-*N*,*N*,*N*-trimethyl ammonium bromide are transferred into the volumetric flask. Subsequent to intermixture and cooling down, it is filled up with distilled water up to the calibration mark. The solution is stored in the dark at 18–20 °C.

Execution of the ADF analysis

Subsequent to the drying at 105 °C, the empty weight of the Fibrebags is determined and recorded (m_1). Then, approximately 1 g of dried sample must be weighed in accurate to 0.1 mg. The mass of the sample in the Fibrebag must be recorded (m_2). A glass spacer is carefully inserted into the Fibrebags and both are placed in the sample carousel. All Fibrebags are thoroughly rinsed with hexane. This way, excess fat is eluated from the samples. The duration and sequence of the process steps of the Fibretherm FT 12 can be found in Tab. 4.11-1.

After completion of the method, the Fibrebags must be dried in the drying cabinet over night at 105 °C and the mass must be recorded (m_4).

If ADL (crude lignin) is to be determined, the "Execution of ADL analysis" must be carried out thereafter. If lignin does not need to be determined, at this point the ashing in the muffle furnace is carried out at 500 °C for at least 2 h. Subsequent to cooling down of the sample in the desiccator, the sample is weighed and the weight is recorded (m_5). The ash determination obtained here is, for the most part, identical to the ash determination from the TS/VS determination (Ch. 3.1).

Execution of the ADL analysis

In preparation, dry crucibles and Fibrebags at 105 °C for 24 h. For the ADL determination, additionally the Fibrebags weighed for the determination of the ADF (prior to the ashing!) are hung in a sample carousel and secured. Subsequently, the sample carousel with the Fibrebags is place in a 5 L beaker and covered at room temperature with 72 % sulphuric acid. The sulphuric acid is stirred every hour and during this period is kept for 3 h at a temperature of 20–23 °C. Subsequently, it is rinsed with hot water to the neutral point and dried for 24 h at 105 °C (m_7).

Table 4.11-1: Method for the determination of ADF

1	Dosage	ADF solution	1.3 L
2	Heating	34 % (device-dependent)	1 h
3	Suctioning off		2 min/30 s
4	Washing cycle 1/2		
5	Washing cycle 2/2		
6	Dosage	H_2O wash	1.3 L
7	Heating	50 % (device-dependent)	0 h 5 min
8	Cooling	90 > 60 °C	
9	Suctioning off		2.5 L
10	Dosage	H_2O wash	1.3 L
13	Heating	55 % (device-dependent)	0 h 2 min
14	Cooling	90 > 60 °C	
15	Method completed		

The ashing of the Fibrebags is carried out at 500 °C for at least 2 h in the muffle furnace. Subsequent to cooling down in the desiccator, the samples are weighed out and the mass is recorded (m_5). In addition, the dry matter of the analysis sample must be determined in order to be able to put the result in relation to the total solids.

Result calculation

$$\mathrm{ADF} = \frac{(m_4 - m_1) - \left(m_5 - (m_6 - m_3)\right)}{\left((m_2 - m_1) \cdot TS_{md}\right)} \cdot 100 \cdot 100 \qquad (13)$$

ADF	Share of acid detergent fibre	$\%_{TS}$
m_1	Mass of the empty dried Fibrebag	g
m_2	Mass of the dried Fibrebag with sample	g
m_3	Mass of the empty crucible of the blank reading	g
m_4	Mass of the crucible & Fibrebag & sample after drying	g
m_5	Mass of the crucible & Fibrebag & sample after calcination	g
m_6	Mass of the crucible & Fibrebag after calcination of the blank reading	g
TS_{md}	Total solids of the dried and milled sample	%

$$\mathrm{ADL} = \frac{(m_7 - m_1) - \left(m_5 - (m_6 - m_3)\right)}{\left((m_2 - m_1) \cdot TS_{md}\right)} \cdot 100 \cdot 100 \qquad (14)$$

ADL	Share of acid detergent lignin	$\%_{TS}$
m_7	Mass of the ADL-crucible & Fibrebag after drying	g

4.12 Determination of Neutral Detergent Fibre (NDF)

Michael Goldstein, DBFZ

Status	The method is an in-house method that is carried out modelled after the prescribed method of VDLUFA, Book of Methods III, 2nd Supplement, Hamburg 1988 (VDLUFA 1988).
Associated standard	Determination of NDF, official method
Area of application of the method	For the determination of components insoluble in neutral detergent solution

By boiling the dried samples in neutral NDF solution, hemicellulose, cellulose, lignin and lignin-N-compounds are not eluated from the feedstuff. This undissolved residue is weighed out after drying and turned to ash. The difference between the ash content and the undissolved residue is referred to as neutral detergent fibre (NDF). Particular attention must be paid to the adherence to the pH value.

Devices and chemicals

- fibretherm FT 12 device, fibrebag (NDF) & accessories
- drying cabinet and Muffle furnace
- precision scale, crucible & desiccator
- NDF solution
- hexane
- aqua dist.

Preparation of the analysis

The samples must be ground to ≤ 1 mm and dried in the drying cabinet at 105 °C for approximately 24 h. Furthermore, for each sample a crucible must be calcinied empty at 500 °C for 2 h. A double determination is carried out. In addition, corresponding to the number of samples, Fibrebags must be dried in the drying cabinet at 105 °C for 1 h.

Manufacturing of detergents for the NDF determination

Devices and chemicals

- 5 L volumetric flask
- 5 L & 1.5 L beaker
- glass funnel
- 50 mL volumetric bulb pipette
- top unit scale and small weighing bowl(s)
- magnetic stirrer with magnetic stir bar
- aqua dist.
- EDTA disodium salt (EDTA disodium salt dihydrate also possible) p. a.

- disodium tetraborate decahydrate p. a.
- dodecylsulphate sodium salt p. a.
- triethylene glycol p. a.
- sodium dihydrogen phosphate p. a.
- soda lye/sulphuric acid p. a.
- antifoaming agent (TANAFOAM 1573)

Manufacturing of the NDF solution

Approximately 2 L distilled water and a magnetic stirrer are placed in a 5 L beaker. 93 g (103 g EDTA disodium salt dihydrate) and 34 g disodium tetraborate decahydrate are transferred into the 5 L beaker. The solution is stirred on the stirring disk until all solids have been dissolved. Subsequently, 150 g dodecylsulphate sodium salt is added into the beaker in the same manner and 50 mL triethylene glycol are pipetted in while stirring.

Approximately 1 L distilled water is placed in a 1.5 L beaker and – while stirring until complete dissolution – 22.8 g sodium dihydrogen phosphate are added into the beaker: Thereafter, this phosphate solution in the 5 L beaker is filled up with distilled water to approximately 4.5 L and 2 mL of antifoaming agent is added. The pH value is measured and adjusted with soda lye/sulphuric acid to be between 6.9 and 7.1. The solution is transferred into the 5 L volumetric flask by means of the glass funnel and filled up to the calibration mark with distilled water. The shelf life of the solution is four weeks.

Execution of the analysis

Subsequent to the drying, the empty weight of the Fibrebags is determined (m_1) and approximately 1 g of dried sample is weighed in accurate to 0.1 mg. The mass of the Fibrebag filled with the sample is recorded (m_2). A glass spacer is carefully inserted into the Fibrebags and both together are placed in the sample carousel. All Fibrebags are thoroughly rinsed with hexane. This way, excess fat is eluated from the samples. After drying for approx. 2 min in the exhaust, the Fibretherm is started with the settings listed in Tab. 4.12-1.

Once the method has been completed, the spacer is removed from each Fibrebag, whereupon care must be taken that none of the samples are discharged. The Fibrebag is placed in the crucible rolled up and dried for approximately 24 h at 105 °C. Subsequent to the drying, it is left to cool down in the desiccator and the mass is determined. The ashing of the Fibrebags is carried out at 500 °C for at least 2 h. After cooling down in the desiccator, the samples are weighed. In addition, the dry matter of the analysis sample must be determined in order to be able to put the result in relation to the total solids.

Result calculation

$$NDF = \frac{(m_4 - m_1) - \left(m_5 - (m_6 - m_3)\right)}{\left((m_2 - m_1) \cdot TS_{md}\right)} \cdot 100 \cdot 100 \qquad 15$$

NDF	Share of neutral detergent fibre	$\%_{TS}$
m_1	Mass of the empty dried Fibrebag	g
m_2	Mass of the dried Fibrebag with sample	g
m_3	Mass of the empty crucible of the blank reading	g
m_4	Mass of the crucible & Fibrebag & sample after drying	g
m_5	Mass of the crucible & Fibrebag & sample after calcination	g
m_6	Mass of the crucible & Fibrebag after calcination of the blank reading	g
TS_{md}	Total solids of the dried and milled sample	%

Table 4.12-1: Method for the determination of NDF

1	Dosage	NDF solution	1.3 L
2	Heating	35 %	1 h
3	Suctioning off		2 min/30 s
4	Washing cycle 1/2		
5	Washing cycle 2/2		
6	Dosage	H_2O wash	1.3 L
7	Heating	55 %	0 h 5 min
8	Cooling	91 > 60 °C	
9	Dosage	H_2O wash	1.3 L
10	Heating	55 %	0 h 2 min
11	Cooling	90 > 60 °C	
12	Method completed		