SLOVENSKI STANDARD
SIST-TP CEN/TR 17739:2022

01-marec-2022

Alge in izdelki iz alg - Specifikacije za uporabo v kemijskem in bioenergetskem sektorju

Algae and algae products - Specifications for chemicals and biofuels sector applications

Algen und algenbasierte Produkte - Spezifikationen für Anwendungen im Chemie- und Biokraftstoffsektor

Algues et produits d'algues - Spécifications pour les applications dans le secteur de la chimie et de la bioénergie

Ta slovenski standard je istoveten z: **CEN/TR 17739:2021**

ICS:

13.020.55	Biološki izdelki	Biobased products

SIST-TP CEN/TR 17739:2022 **en,fr,de**

TECHNICAL REPORT

RAPPORT TECHNIQUE

TECHNISCHER BERICHT

CEN/TR 17739

December 2021

ICS 13.020.55

English Version

Algae and algae products - Specifications for chemicals and biofuels sector applications

Algues et produits d'algues - Spécifications pour les applications dans le secteur de la chimie et de la bioénergie

Algen und algenbasierte Produkte - Spezifikationen für Anwendungen im Chemie- und Biokraftstoffsektor

This Technical Report was approved by CEN on 5 December 2021. It has been drawn up by the Technical Committee CEN/TC 454.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.

EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

Ref. No. CEN/TR 17739:2021 E

Contents

Page

European foreword

This document (CEN/TR 17739:2021) has been prepared by Technical Committee CEN/TC 454 "Algae and algae products", the secretariat of which is held by NEN.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

The European committee for Standardisation (CEN) was requested by the European Commission (EC) to draft European standards or European standardisation deliverables to support the implementation of Article 3 of Directive 2009/28/EC for algae and algae products or intermediates. The request presented as Mandate M/547, also contributes to the Communication on "Innovating for Sustainable Growth: A Bio economy for Europe".

The former working group CEN Technical Board Working Group 218 "Algae" was created in 2016 to develop a work programme as part of the Mandate. The technical committee CEN/ TC 454 "Algae and algae products" was established to carry out the work program the secretariat of which is held by NEN. CEN TC 454 set up a number of topic specific working Groups listed below to develop standards for algae and algae products.

This document has been prepared by Working Group 5 "Specifications for the chemicals and fuels applications sector" with the support of UNI as the secretariat, in close collaboration with the other CEN TC 454 working groups:

CEN TC 454 WG 1 "Terminology"

CEN TC 454 WG 2 "Identification"

CEN TC 454 WG 3 "Productivity"

CEN TC 454 WG 4 "Specifications for food/feed sectors applications"

CEN TC 454 WG 6 "Product test methods"

The interest in algae and algae-based products or intermediates as a renewable and sustainable source of carbohydrates, proteins, lipids and pigments has increased significantly in Europe.

Algae-based products and intermediates, in this TR referred to as 'products', are defined as whole biomass, extracts or derivatives from algae, including algae oil and algal meal.

This document will allow the stakeholders to have access to a clear point of reference on the use of algae in technical applications.

Algae as raw materials have specific challenges and show a wide potential from sustainability and blue economy point of view. This document aims helping to fill this gap.

Any feedback and questions on this document should be directed to the users' national standards body. A complete listing of these bodies can be found on the CEN website.

According to the CEN/CENELEC Internal Regulations, the national standards organisations of the following countries are bound to announce this Technical Specification: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Introduction

The interest in algae and algae-based products or intermediates as a renewable and sustainable source of carbohydrates, proteins, lipids and pigments has increased significantly in Europe.

The biochemical composition of algae with regard to the ratio of usable components is unique and the selected conversion pathway determines the kind of products that can be obtained.

Algae are available and used in many countries as fertiliser, biostimulant, animal feed, medicine, cosmetic and food ingredients, and can provide different compounds depending on species. Due to the high interest in replacing fossil feedstock for chemicals and fuels by biological ones, as algae, several pilot initiatives have been undertaken in last decades. Results strongly vary, as many techno-economic parameters are expected to influence the success of an algae related initiative.

Cultivation of algae has advantages over other sources of biomass, since they can be cultivated on marginal lands and unused aquatic areas, generating new economic opportunities for poor soil and aquatic areas, which would not have traditionally been used.

Due to their relatively simple cellular structure, algae have a large biomass productivity per unit area. Algae are of great ecological importance, since they act in the capture of atmospheric CO_2 and can be used as wastewater treatment, due to their capacity to remove nutrients, as their growth requires sunlight, water, CO_2 and nutrients.

1 Scope

The purpose of this document is to provide an overview on how quality indicating parameters for algae and algae products and intermediates relevant for chemical and bioenergy applications can be handled and to identify the need for future standards development for chemicals, bioenergy and biofuels applications.

This document does not provide instructions on handling of technical requirements in existing legislations.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 17399:2020, *Algae and algae products - Terms and definitions*

EN 14214, *Liquid petroleum products - Fatty acid methyl esters (FAME) for use in diesel engines and heating applications - Requirements and test methods*

EN 590, *Automotive fuels - Diesel - Requirements and test methods*

EN 16734, *Automotive fuels - Automotive B10 diesel fuel - Requirements and test methods*

EN 16709, *Automotive fuels - High FAME diesel fuel (B20 and B30) - Requirements and test methods*

EN 16723-1, *Natural gas and biomethane for use in transport and biomethane for injection in the natural gas network - Part 1: Specifications for biomethane for injection in the natural gas network*

EN 14103, *Fat and oil derivatives - Fatty Acid Methyl Esters (FAME) - Determination of ester and linolenic acid methyl ester contents*

EN 17477, *Algae and algae products - Identification of the biomass of microalgae, macroalgae, cyanobacteria and Labyrithulomycetes - Detection and identification with morphological and/or molecular methods*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN 17399:2020 and the following apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- IEC Electropedia: available at https://www.electropedia.org/
- ISO Online browsing platform: available at https://www.iso.org/obp

3.1
Raw Material Specification
RMS
technical dossier, several pages long, about the product, usually prepared by manufacturer, directed to provide all product approval information to the customer and usually attached to commercial contract

Note 1 to entry: Examples of models of RMS for some algae categories are reported in Annex A.

3.2
Technical Data Sheet
TDS
technical document, one (or few) page long, showing the technical (bio- and physicochemical) parameters adopted to characterize the product and therefore being the paradigm of the Certificate of Analysis (CoA)

Note 1 to entry: It includes ranges of different parameters used to define the product characteristics or applicable regulatory limits.

Note 2 to entry: Examples of Models of TDS for some algae categories are reported in Annex B.

Note 3 to entry: In cases where the producer wants to make statements regarding the algal product as a bio-based product EN 16848 and EN 16935 should be used.

3.3
Certificate of Analysis
CoA
one (or few) page document issued from a laboratory (or laboratories) and reporting test results for a specific lot, usually in front of TDS parameters, including references to test method

Note 1 to entry: It may or may not have legal value.

3.4
Material Safety Data Sheet
MSDS
SDS
document issued with the aim of providing information about product compliance in respect of human health and safety at the workplace and protection of the environment

Note 1 to entry: The MSDS should comply with Reg 1907/2006 (REACH).

3.5
sustainable development
development that meets the environmental, social and economic needs of the present without compromising the ability of future generations to meet their own needs

[SOURCE: ISO Guide 82:2019, definition 3.2]

3.6
extracts
products of liquid, solid or intermediate consistency, obtained from algal biomass and containing components present in and/or derived from the algal biomass

Note 1 to entry: For some preparations, the biomass to be extracted undergoes a preliminary treatment, for example, inactivation of enzymes, grinding or freezing.

3.7
energy from renewable sources
energy from renewable non-fossil sources, namely wind, solar (solar thermal and solar photovoltaic) and geothermal energy, ambient energy, tide, wave and other ocean energy, hydropower, biomass, landfill gas, sewage treatment plant gas and biogas

[SOURCE: Directive (EU) 2018/2001 of the European Parliament and of the Council of 11 December 2018 on the promotion of the use of energy from renewable sources (recast) [6]]

3.8
bioliquids
liquid fuel for energy purposes other than for transport, including electricity and heating and cooling, produced from biomass

[SOURCE: Directive (EU) 2018/2001, ibid. [6]]

3.9
biofuels
liquid fuel for transport produced from biomass

[SOURCE: Directive (EU) 2018/2001, ibid. [6]]

3.10
advanced biofuels
biofuels which technology is more innovative and less mature that are produced from feedstock which has low indirect land-use change impacts

Note 1 to entry: this feedstock includes algae if cultivated on land in ponds or photobioreactors.

[SOURCE: Directive (EU) 2018/2001, ibid. [6]]

3.11
biomass fuels
gaseous and solid fuels produced from biomass

[SOURCE: Directive (EU) 2018/2001, ibid. [6]]

3.12
biogas
gaseous fuels produced from biomass

[SOURCE: Directive (EU) 2018/2001, ibid. [6]]

3.13
guarantee of origin
an electronic document which has the sole function of providing evidence to a final customer that a given share or quantity of energy was produced from renewable sources

[SOURCE: Directive (EU) 2018/2001, ibid. [6]]

3.14
low indirect land-use change-risk biofuels, bioliquids and biomass fuels
biofuels, bioliquids and biomass fuels, the feedstock of which was produced within schemes which avoid displacement effects of food and feed-crop based biofuels, bioliquids and biomass fuels through improved agricultural practices as well as through the cultivation of crops on areas which were previously not used for cultivation of crops

Note 1 to entry: Crops which were produced in accordance with the sustainability criteria for biofuels, bioliquids and biomass fuels laid down in Article 29 of Directive (EU) 2018/2001.

[SOURCE: Directive (EU) 2018/2001, ibid. [6]]

4 Algae products applications as chemical raw materials

4.1 General

For multi-constituent natural ingredients, with variable composition, it is essential that the producers provide clearly defined specifications in view of the range of variability of the components.

The variability should not change significantly and minimum levels of active components should be specified.

In cases where the producer wants to make statements regarding the algal product as a bio-based product EN 16848 and EN 16935 should be used.

4.2 Bioplastics from algae

A considerable attention has been paid, in recent years, to alternative feedstock for bioplastics production. Microbial production of exopolysaccharides (EPSs) is an interesting field of research, in view of the increasing demand for natural biofilm products for medical, and industrial applications. Microalgal EPSs have potential antioxidant, antibacterial, and emulsifying activities and may be potentially obtained from direct extraction from algae. Specifically, polymers such as starch or polyhydroxyalkanoates (PHAs), which are already being used in plastic production, are of interest. Starch on the other hand is not a polymer with plastic properties. It can be modified with additives to achieve thermoplastic properties or used as a filler in other plastics. Similarly, saccharides found in algae can be used as feedstock for the production of building blocks for bio-based plastic, such as lactic and succinic acid [1] [2] [3].

Moreover, PHAs are widely produced by heterotrophic bacteria. These compounds can replace commercial plastics, such as polyethylene and polypropylene, because of their high biodegradability and biocompatibility. One of the best-studied and commercially available biopolymers is poly-b-hydroxybutyrate (PHB) see Figure 1, which constitutes the intracellular components of microbial cells, and that can be accumulated in significant amount under specific growth conditions. PHAs possess inherent thermoplastic properties and will not need to be modified to create a plastic film.

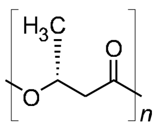

Figure 1 — Chemical structure of P3HB

Properties will depend on the exact structure of the PHA, the purity of the extracted material, molecular weight and the extracted amount of PHAs.

However, if a plastic is produced with PHAs and additives or fillers the degradation process can become more complex.

Life-Cycle analysis has been a valuable tool to assess the environmental impact of bio-plastics along their life cycle. Taking into account the various challenges of this field and the constant development, a specific methodology was proposed in order to assess the potential environmental impacts of the use of alternative feedstocks (biomass, recycled plastics, CO_2) in comparison to current feedstocks (oil and gas) [4].

EN 16760 "Bio-based products – Life cycle assessment" is applicable for bioplastics produced from algae, see also clause 8.

Beside LCA issues, methodology for assessing standards for alternative feedstock for bioplastic production is a field still under development. The current production relies on few feedstocks, and there is a need to enlarge the basket of potential alternatives, if the sector aims to continue on the current growth.

It could be of interest to focus the analysis of the current legislative framework on the availability of quality standards for alternative feedstock, including algae as potential interesting and widely available source.

4.3 Fertilisers

Fertilisers are defined and regulated by Reg 1009/2019 EU (Fertiliser Product Regulation, FPR) [5]. Whereas microalgae only recently got this application due to high cost, and mainly as biostimulants, seaweeds have long history of use as fertilisers since 3000 BC thanks to availability from wild gathering, which action improved the sea life environment and helped to prevent the accumulation of dead seaweed on the shores and in the deep sea.

According to FPR, algae are included together with plants as i) raw material for EU fertiliser product or ii) input material for the production of biogas in anaerobic fermenters, from which the digestate can be contained in EU fertiliser product. However, in both cases cyanobacteria ("blue-green algae") are excluded; this gap excludes actually *Arthospira sp.* (Spirulina) from EU fertiliser and might be fixed by appropriate amendment of FPR, e.g. inclusion of safe cyanobacteria in microorganism category. More information about FPR is given in Annex C.

4.4 Biostimulants

Plant biostimulants are substances, mixtures and micro-organisms which, differently from straight fertilisers, are not as such inputs of nutrients, but nevertheless stimulate plants' natural nutrition processes. They act in addition to fertilisers, with the aim of optimising the efficiency of those fertilisers and reducing the nutrient application rates and are by nature more similar to fertilising products than to most categories of plant protection products. Such products are therefore eligible for CE marking under Reg 1009/2019 and excluded from the scope of Reg (EC) No 1107/2009 on Plant protection products (e.g. pesticides).

Plant biostimulants constitute Product Function Category (PFC) 6 of fertilisers, products made to stimulate plant nutrition processes independently of the product's nutrient content with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere:

a) nutrient use efficiency;

b) tolerance to abiotic stress;

c) quality traits; or

d) availability of confined nutrients in the soil or rhizosphere.

An example of main product requirements and conformity assessment for a plant biostimulant composed of algae-based material (under FPR) to be marketed as EU fertiliser is given in Annex D.

A table of regulated contaminants filled from Reg 1009/2019 EU is given in Annex E.

5 Biofuels from algae

5.1 General

The product characteristics specified from clause 5.2 to clause 5.5 should comply with the relevant standards and regulations.

In case the product characteristics are referred to algae and algae products raw materials, existing standards are referenced when applicable and recommendations for possible developments provided.

In the current worldwide energy matrix, there is a strong dependence on non-renewable energy sources, mainly coal, oil and natural gas. Considering the production of sustainable energy sources, the use of biofuels has become an important alternative, due to a reduction in CO_2 emissions into the atmosphere. An overview on algae biofuels literature is given in Annex G.

Biomass production aimed for biofuels has general sustainability standards: ISO 13065 as well as EN 16214-1, EN 16214-3 and EN 16214-4, where the first is applicable to all types of bioenergy.

Concerning liquid biofuels, it is usual to classify them as first, second and third generation. The most well-known first-generation biofuel is ethanol, extracted from the fermentation of sugar and starch present in plants in addition to biodiesel produced from oilseed plants. Second-generation biofuels are those produced from the processing of the lignocellulosic fraction of biomass from higher plants. Biofuels from algae are often mentioned as third generation.

Algae are explicitly mentioned in The Renewable Energy Directive recast (RED II) [6] as feedstock for the production of biogas for transport and advanced biofuels, and can be counted twice if cultivated on land in ponds or photobioreactors. Additionally, algae are listed in RED II as feedstock that can be processed only with advanced technologies. Feedstock that can be processed into biofuels, or biogas for transport, with mature technologies, such as used cooking oils and animal fats, have a share limited to 1,7% (energy content).

Bioethanol, biohydrogen, biodiesel and biogas (biomethane) have been proposed from algae through processes such as fermentation, dark fermentation/photobiological production, lipid extraction/transesterification and anaerobic digestion, respectively. On the other hand, different thermochemical processes have been proposed for algae, such as pyrolysis, gasification, torrefaction and hydrothermal liquefaction, depending on process conditions (pressure, temperature, reaction time, oxygen availability and feedstock water content, among others). Thermochemical conversion processes yield bio-oil (which can be eventually converted in drop-in liquid biofuels), biogas/syngas and char. There is still need for algae-based process development and intensification in order to make full use of the wide range of bio-based products, biofuels and environmental services algae and algae products can generate (such as CO_2 and other GHG biosequestration, waste water treatment – phycoremediation- and heavy metals tertiary treatment, among others). Besides bioenergy production from algae, a wide range of commercially valuable bio-based products/materials and/or high added value products can be extracted, fractionated and purified, following the biorefinery approach. For commercial viability, a matrix approach leading to numerous products is preferred for successful operation of algal biorefineries.

5.2 Fuel specifications

Fuels are characterized by energy density and technical parameters related to engine suitability (e.g. cetane or octane number, impurities, ash content, oxidation stability, etc.).

No algae fuels are currently present in the market even if several Institutions (EU Commission, U.S. Department of Energy) are interested and several Companies are working on that topic. According to available literature, algae derived fuel feedstock allow for entering in already developed conversion pathways, e.g. gaseous fuels like biomethane, and liquid fuels for diesel substitution like biodiesel or alternative fuels for aviation sector.

It is worth to note that algae are considered for their photosynthetic potential as alternatives to terrestrial plants (the actual main source of biodiesel, hydrogenated vegetable oil (HVO) and biogas besides waste products) whereby the carbon-based molecule is biosynthesized converting solar energy in chemical energy. Algae have neither to be considered as an intermediate nor as a finished fuel, but as a raw material for certified fuels production, therefore no algae specific standards have to be mentioned in this section.

NOTE The only relevant exemption is represented by the aviation sector. In aviation not only the final fuel, but also the conversion process has to be fully certified.

The American Society for Testing and Materials (ASTM) issued two technical norms regulating the sector: ASTM D4054 (Standard Practice for Qualification and Approval of New Aviation Turbine Fuels and Fuel Additives), which describes the qualification process for an alternative fuel to be considered compliant for use in ASTM D7566– 17a (Standard Specification for Aviation Turbine Fuel Containing Synthesized Hydrocarbons).

Currently eight production pathways are already fully certified for blending with fossil aviation jet fuel. These aviation biofuels are drop-in fuels: they can be directly blended with fossil (ASTM D1655: Specification for Aviation Turbine Fuels) but with different blending limits.

At the time of writing, no specific algae-based pathway results certified for aviation [7].

5.3 Liquid biofuels from algae

5.3.1 General

A first categorisation of liquid fuels can be performed on the base of their possibility to be directly mixed to regular fuel without specific limitations. The term "drop-in" fuel is generally used to describe an alternative fuel, that can be blended into a regular fuel, without compromising any functionality of the engine. Hydrogenated Vegetable Oil (HVO) is usually recognised as a drop-in fuel, while biodiesel and bioethanol are not.

The final characteristics of the alternative fuels, which derive from the feedstock/conversion process couple, determines whether the final product can be considered a drop-in fuel or has to respect a blending limit.

Liquid fuels from algae currently under development can be divided in two main groups, e.g. fuels derived from algae oil (biodiesel, HVO) and alcohols, usually ethanol, obtained by fermentation of algae carbohydrates. In principle, alcohols and other algae derived molecules can be used to synthesize drop-in liquid fuels (i.e. Fischer-Tropsch fuels from algal biomethane, jet fuel from algal alcohols).

5.3.2 Product characteristics of liquid fuels from algae lipids

5.3.2.1 General

The lipid content in microalgae is very diverse. The cell structure, cell wall characteristics, the lipid binding with proteins and carbohydrates and lipids for energy storage vary from species to species and even within species depending on cultivation (culture age, growth phase and nutrient availability) or extraction condition. The extraction methods will affect not only the total extraction yield, but, in some cases, the fatty acid profile, especially when extraction yield is low. Therefore, a general recommendation on a specific algae or extraction method cannot be given. However, heterotrophic algae and phototrophic algae under starvation (metabolic induction, e.g. by nitrogen depletion in the last culture phase) are known to have increased lipid content.

5.3.2.2 Algae lipids

Algae lipids are defined as class of natural organic substances characterized by very low water solubility, high solubility in organic solvents, high carbon and hydrogen content, biosynthesized for energy storage and/or metabolic and structural functions. Several test methods for total lipids content of marine tissues were applied to algae since the fifties (Bligh&Dyer [8], Folch [9], Smedes [10]) ending in the highly recommended need for a standard, which is currently developed in CEN/TC 454, see below, and the one described in clause 6.7.

5.3.2.3 Algae oil

Algae oil is defined as glyceridic fraction of lipids derived from algae (EN 17399:2020, 3.2).

The detailed composition and molecular profile of lipids is required for reporting on oil quality and biomass valorisation and will be highly influential when targeting particular bioproduct markets, for example for biodiesel or green diesel. Not all lipids can be considered equally valuable for fuel or even food or feed applications. The lipid composition, with respect to polar (phospho- and glycolipids) and non-polar (triglycerides and sterols) lipids and the respective impurities found in each fraction, is highly dependent on the origin and type of biomass. Autotrophically grown algae are rich in polar lipids, waxes, sterols, and pigments, whereas heterotrophic cultivation will yield triglyceride-rich oil similar to plant-derived oils, but often with very different fatty-acid profiles. Traditionally, lipids have been measured gravimetrically after solvent extraction. The completeness of extraction depends on composition, therefore on the biochemistry of the alga and on the recent physiological conditions experienced by the organism. Therefore the extraction solvent polarity must cope with the lipid molecule polarity and the extraction conditions used, in order to avoid inconsistent lipid yields. Inevitably, the extractable oil fraction will contain non-fuel components (e.g. chlorophyll, other pigments, proteins, and soluble carbohydrates). Thus, it may be very useful to assess its fuel fraction (i.e. fatty acid content) by transesterification followed by quantification of the fatty acid methyl esters (FAMEs). However, due to the large number of variables, it is necessary to standardize the extraction-based total lipid quantification procedure [11] in order to be in position to refer the fatty acids content to lipids and not only to whole biomass. There are two extraction systems currently in use across analytical laboratories: conventional Soxhlet extractor systems and the Randall extraction systems with the more recently developed pressurized fluid extraction systems. However modified Bligh-Dyer [13] or Smedes [10] dual solvents extraction method were recommended for complex matrixes of marine origin such as fish tissue [11] [12]. Since most microalgae and seaweeds are marine organisms and show lipids patterns similar to fish tissue and very different from terrestrial oilseeds, a more powerful extraction system than Soxhlet and its variations is necessary. Immersion extraction and sonication over freeze dried test portions provided best accuracy and precision with appropriate and well standardized protocol which was demonstrated to work also with algae showing composition similar to oilseeds, such as heterotrophs. [13]. The basic principles of such protocol are: 1) to extract quantitatively all lipids by proper mixed solvent (chloroform/methanol), high solvent/solid ratio and multiple washes, 2) to purify the extract by removal of non-lipids fractions through liquid-liquid purification. For certain microalgae with a very hard cell wall, a pre-treatment of bead beating is provided.

This protocol is going to become the new standard as the reference test method for total lipid content.

As an alternative to extraction, for routinary purposes of multiple testing of the same alga there is a growing emphasis on the quantification of lipids through a direct (or in situ) transesterification of whole algal biomass and to application of rapid indirect measurement of lipids by near infrared spectroscopy (NIR) and nuclear resonance spectroscopy (NMR).

However this methods have two major drawbacks: 1) the need for case by case calibration by absolute method (e.g. extraction and gravimetry) and 2) the need of sophisticated and expensive apparatuses.

Finally, qualitative or semi-quantitative (after calibration) lipids data can be gathered through high-throughput methodologies that are based on hydrophobic (lipophilic) fluorescent dyes such as Nile Red [14].

While fatty acids measurement by direct transesterification and gas chromatography (GC) cannot substitute extraction for total lipids, it is a powerful tool for algae oil quality characterization. The process consists of either a two-step alkaline and subsequent acid hydrolysis of the biomass, or a single-step acid catalysis, followed by the methylation of the fatty acids to fatty acids methyl esters (FAME) and quantification by GC. These procedures have been demonstrated to be robust across species and their efficacy is less dependent on the parameters listed above that influence lipid extraction. However, if the relative composition of intact lipids is required (e.g. polar versus neutral lipid content), an extraction process may be the only way to isolate intact lipids from the rest of the biomass, with the utilization of advanced instrumentation, such as liquid chromatography for the characterization of the lipid molecular profile.

Fatty acid profile and content of vegetable fats and oils can be measured according the standards of ISO 12966. It is strongly recommended that for fatty acids determination in algae ISO 12966 is examined.

5.3.3 Algae oil for biodiesel

5.3.3.1 General

Algae oil, i.e. fatty acids, either free or bound (on glycerol), could be converted into FAME, also known as biodiesel. The product shall comply with the biodiesel standard EN 14214 for its usage, either pure or as blending component for a diesel fuel according EN 590, EN 16734 or EN 16709.

The share of FAME in mineral diesel is limited in EN 590 to 7,0 %v/v, in EN 16734 to maximum of 10,0 %v/v and in EN 16709 to a maximum of 30,0 %v/v.

Several parameters of EN 14214 are related to the fatty acid profile of the raw material, as they will not be changed significantly in the biodiesel process. As consequence, these parameters should already be fulfilled by the algae oil, or the algae oil should be blended with other fats and oils to meet the parameters.

5.3.3.2 Fatty acid profile

The fatty acid profile related parameters should be fulfilled in the final biodiesel according to EN 14214. As unsaturated fatty acids are vulnerable for oxidation, they are limited by several parameters. Oxidation products can damage the engine [15]. However, due to this oxidizability, biodiesel is more easily biodegradable than fossil fuels.

5.3.3.3 Other chemical parameters of oils

The iodine value is a measure for the content of unsaturated fatty acids. It describes the mass of halogen, expressed as iodine, absorbed by the test portion when determined in accordance with the procedure specified in EN ISO 3961, divided by the mass of the test portion [16]. The limit for the iodine value is maximum 120 g iodine /100 g. The iodine can be measured according to EN ISO 3961 by titration or EN 16300 via the fatty acid profile.

The linolenic acid methyl ester (C18:3) content is limited with maximum 12,0 %(m/m). In the final product it should be measured according EN 14103.

The content of polyunsaturated methyl ester (PUFA) shall be below 1,0 %(m/m), whereas in this case polyunsaturated is defined as “more or equal 4 double bounds” in the fatty acid chain. Its content is measured according EN 15779 which covers the usual predominant four PUFA (C20:4, C20:5, C22:5, C22:6). Short chain PUFA, which might be present in algae oil, e.g. C16:4, are not considered but might have an influence on the oxidation stability of the fuel.

The oxidation stability [15] is related to the fatty acid profile and to the presence of natural antioxidants in the oil. However, if a distillation is required as purification step of the FAME, natural antioxidants will