NIR spectrometry for monitoring of nutritional value of algae

An in-depth review of NIR technology, current applications in the algae field and their relevence for health-care diets

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***Abstract* - Health-care practitioners have suggested microalgae as a food supplement in liquid diets because of their beneficial properties. However, the quality of such a food supplement has to be high in order to meet medical standards. Therefore, monitoring tools capable of determining nutritional values have to be found. This review investigates the feasibility of using near infrared (NIR) spectrometry as a monitor of the nutritional values of microalgae in the cultivation process. NIR is a form of vibrational spectrometry, which can be used for nondestructive and real-time measurements of compounds. Recent applications are discussed that prove the capability of NIR to ensure quality in algae food supplements. These are divided in on-line and off-line monitoring capabilities. On-line applications were preferred because of the possibility of real-time control feedback creating quality insurance. It was found that NIR technology has the potential to determine the nutritional values of algae in an off-line setting. For on-line monitoring however, the disturbance created by water, which overlays important absorbance peaks, was found to be extensive. In the end Raman and FT-ATR spectrometers are briefly discussed, which do not have this setback.**

Keywords-component; NIR; Algae; nutriton

I. INTRODUCTION

Hippocrates, the father of medicine, already knew that a good diet is important for the quick recovery of patients. However, some therapies require special diets, which can restrict a complete nutrition. One of these diets is called the full liquid blenderized diet. This diet provides nourishment without requiring mastication. It is meant for patients with oral esophageal disorders, neuromuscular disabilities, advanced carcinoma of the oral cavity or with facial or neck trauma, whether or not from surgery. In addition, patients who received radiation therapy who have difficulty eating use this diet [2].

Evidence was found for the anti-cancerous properties of this omega 3 lipid acids [3]. Omega 3 is mostly found in seafood. However, patients experience palatability problems in consuming blended fish. In addition, restricting dietary wishes and allergies are on the rise [1], which causes a need for another source of omega 3 lipid acids. Sea animals acquire the Omega 3 fatty acids by accumulation from microalgae. Microalgae have been suggested for use by health-care practitioners because of their beneficial properties in the treatment of cancer, AIDS, some gastrointestinal and digestive disorders, some forms of hypercholesterolemia, malnutrition, infections, pesticide poisonings, heavy metal poisonings, some forms of chemical toxicities and in preventing radiation poisoning [4]. Furthermore, algae are a good source of all basic nutrients like carbo hydrates, proteins and lipids. In addition, algae food supplement are mild in flavor and can easily be added to solutions. For these reasons, algae seem to have potential as a food supplement for patients who are on a liquid diet.

However, in order to be able to implement algae as a food supplement for hospital care, industry standards have to be achieved to ensure quality. At this time, each producer has its own standards, which results in a lack of continuity among different products from different companies. One of the problems for achieving a standard is the variability of the nutritional value of algae. The nutritional value is influenced by many factors in the growth process. Some of these factors are harvesting time, nutrients, temperature, light intensity, specie of algae and the method of culturing [5]. Because most algae are cultivated outside where these factors are subject to unpredictable change, a good monitoring system is required. This system should be able to determine the nutritional values of the algae in the cultivation process with high accuracy. This would open up the possibility for a feedback loop in order to be able to steer the nutrients towards the desired standard values.

Monitoring of bioprocesses on quality, consistency, homogeneity and productivity has been recommended. The European federation on biotechnology (EFB) Section on Biochemical Engineering Science (ESBES) issued guidance for the biopharmaceutical industry under the term ‘Modeling, Monitoring, Measurement & control’ (M3C) [9]. The FDA published similar guidelines on achieving this level of control under the term ‘process analytical technology’ (PAT) [10]. The goal of these methods is to apply high time resolution measurements of reagents in a process for design, analysis and, ultimately, control product quality, in which the product quality monitor becomes built in the process of production

[11]. It can be argued that these design recommendations should be fundamental in any microalgae cultivation design in order to produce a medical grade food supplement. To implement these recommendations, the control over critical process parameters (CPPs) is required while considering critical quality attributes (CQAs) [12]. The CPPs are the factors determining the growth process, while CQAs determine the attributes of the product and should be within a desired range in order to meet the quality and consistency of an improving product. If the CQAs of a product can be estimated, it will allow adjustments of the CPPs in order to better control the final values of the product.

Near-infrared (NIR) spectroscopy is a fast and nondestructive analytical technique that provides chemical and physical information of virtually any matrix [6]. NIR spectroscopy distinguishes itself from other vibrational spectrometers because it scans in the spectrum that contains the overtones and combination bands from the mid infrared (MIR) spectrum [7]. Therefore, the interference caused by the absorbance of water in this region is a thousand fold lower than in the MIR spectrum, making it possible to use water as a solvent for some applications [8]. NIR can be used to monitor CQAs and some CPPs in real-time and is used in many fields of science like agriculture, food, chemistry, pharmaceutical, medicine, environment and geology, to name a few.

This review provides a global understanding of NIR technology, the calibration needed to use it for quantity research and the placement of the sensor in a bioprocess. After discussing the advantages and disadvantages, it investigates the feasibility of using NIR spectrometry as a monitor of the nutritional values of microalga. Specifically, it looks at the possibility to determine the amount of carbohydrates, proteins and lipids by discussing research that has taken place. Favorably, the monitoring capabilities will be present in the cultivation process, otherwise after harvest of the biomass.

II. NIR Technology

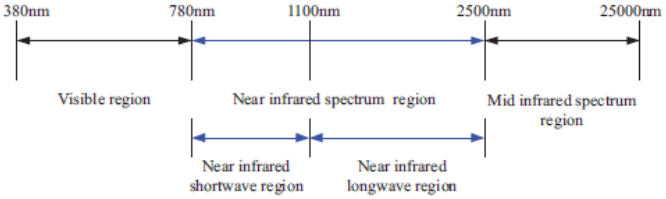

Figure 1. Spectral wavelength distribution diagram, from [28].

NIR is nondestructive and can be used for in-line measurements and thus for real-time control feedback. The NIR region of the electromagnetic spectrum lies in between 780 and 2526 nm wavelength (see Fig. 1). Molecules exposed to this energy range will take up the photon energy and transit into their lowest excited vibrational state in the form of stretching or bending their hydrogen bond. Once the molecule falls back in its inactive state, it will admit this energy again. This varies for different frequencies of radiation, because the molecular bonds of the molecules in the sample have different vibrational frequencies, and thus can absorb different light frequencies. The difference in light intensity is measured before and after interaction with the target, which indicates the amount of absorbance. This can be done in six measuring modes (see Fig. 2).

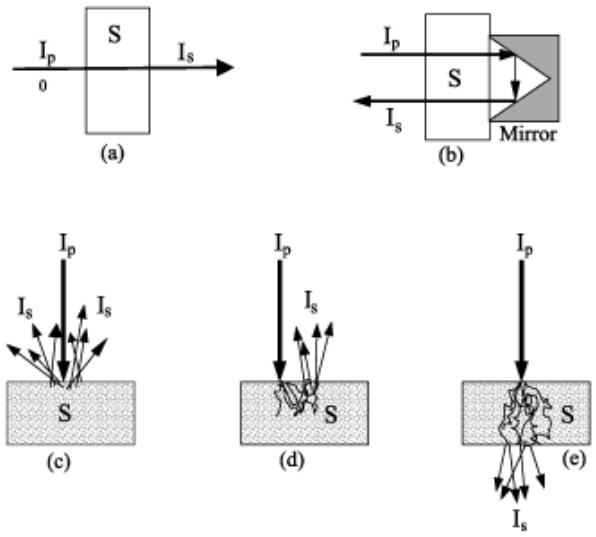

Figure 2. Modes of measurement employed in NIR spectroscopy. a, transmittance; b, transflectance; c, diffuse reflectance; d, interactance, and e, transmittance through scattering medium, from [7].

Transmittance, seen in Fig 2 a, operates with the same principle as conventional UV-VIS spectrometry. By measuring the absorbance of light by the solution, a linear relation with concentration can be gained. This is called Beer's law. The coefficients in this equation are unique for each substance. Analyzing the relation allows for qualitative and quantitative assessments of the substances present in the solution. Fig 2 b shows transflectance, which operates in the same way as transmittance, but the optical pathway is doubled. The diffuse reflectance, as seen Fig 2 c, is the combination of scattering and absorbance, which is unique for each substance. In the interactance mode, as seen in Fig 2 d, the assumption has been made that the incident beam will have a high interaction with the substance. The emerging beam located distant from the incidence beam will therefor hold more information about the composition of the substance. The same principle holds for the transmittance through the scattering medium as seen in Fig 2 e. Combinations and overtones of radiation-molecule interactions dominate the NIR spectrum. Especially the O-H, C-H, S-H and N-H bonds result in NIR absorbance. Because these bonds all share the hydrogen atom, overlaps occur [15]. This leads to broad peaks and hard to interpret data compared to other vibrational spectrometers. As a result, it can be difficult to determine which chemical components are present and how they affect the reflectance. That is why careful calibration with large sample sets becomes key in order to use NIR for specific applications.

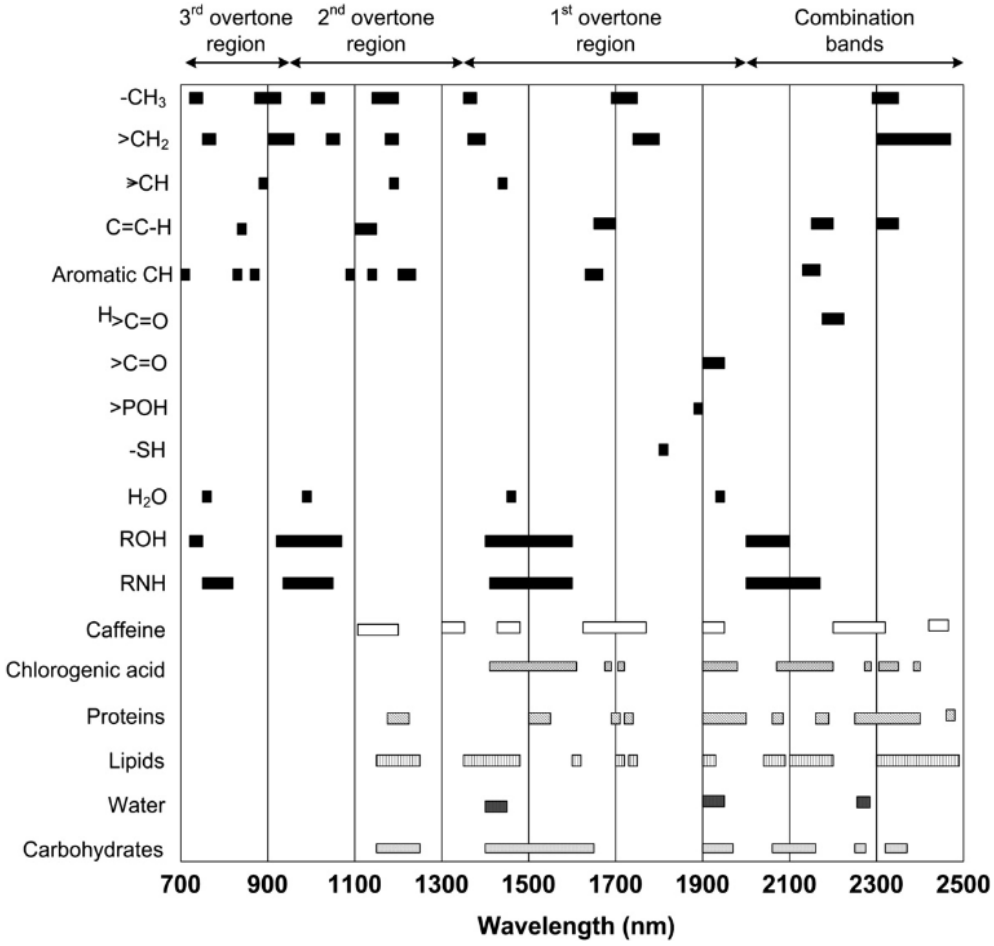

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Figure 3. Principal types of NIR absorption bands, their locations and their corresponding nutritional compound [42].

III. CALIBRATION

In order to go from absorption spectra to nutritional values, chemometrics is needed. Chemometrics are the statistical methods applied to interpret NIR spectra in order to determine its underlying components. The most common used technique is Partial least square regression (PLS) [16]. However, if it is applied to a complex sample consisting of many components, interference may occur. Water is a common interferer because its hydrogen bonds are quite similar to other interesting bonds lying in the NIR spectra. To minimize these effects, good calibration models are needed. A good calibration model only uses certain informative parts of the NIR region in order to determine which components of interest are present in the sample. This can improve the performance of techniques like PLS in order to use it for applications in water [27]. Several of these techniques are used today.

Once the performance is optimized, the actual nutritional values can be measured. This is done by interpreting which bonds are most commonly present in the sample.

Fig. 3 shows which wavelength are associated with which bonds [42]. These bonds come back throughout the whole NIR spectrum in the form of combinations and overtones. The coincidence of the water bonds with the bonds representing protein, lipids and carbohydrates is unenviable. This is one of the reasons why it is found troublesome to interpret NIR data of aqueous solutions. In general, C-H bonds can be related to carbohydrates and lipids, N-H bonds to protein and O-H bonds to water.

IV. SENSOR PLACEMENT

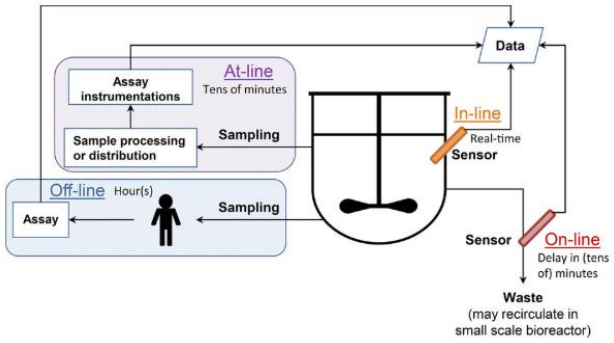

Figure 4. Different types of measurements in bioprocess [13].

The placement of a sensor is determined as either off-line, at-line, on-line or in-line (See Fig. 4 [13). In-line is the most desired situation. It gives constant real-time feedback, which allows for closed loop process control. In a batch process, this would mean that the sensor would be in the bioreactor. In a continuous process, it would mean the sensor can only measure at a single point, or its range has to be prolonged by the use an of optical fiber cable [13].

On-line measurements require detraction of a sample stream from the cultivation process. This stream may recirculate back in, or may be disposed. This also allows closed loop control, but does have a delay in measuring the CQAs compared to in-line, resulting in a postponed response on the CPPs.

At-line and off-line both require manual sampling and sometimes even sample preparation. This happens less often which results in less data and rough feedback. A common technique in algae studies that is classified as off-line is freeze-drying/lyophilization. Another common term is in-situ. This is defined as in cell cultivation medium, which is classified as in-line.

For controlling the nutritional values of the microalgae, an in-line measurement device is preferred for its real-time attributes [13].

V. Other attributes of NIR

Recent progress in NIR technology increased its practicality due to improving sensibility, portability and low production and energy costs [16]. This has led to the use of NIR as a turbidimetric tool to estimate the microalgae density to optimize production [17].

The main reason why NIR has been selected is its ability to do fast nondestructive in-situ analysis of components without sample preparation. Another advantage of NIR is that it can penetrate much deeper into a sample than other vibrational spectrometers and it is not affected by fluorescence [14].

Besides monitoring of chemical components, NIR can monitor other factors as well. It was found that NIR is capable of determining the pH and osmolality [36]. Another study proved its capability to predict the total cell count and the amount of glucose (determines the effectiveness of the growth medium) in a mammalian cell bioreactor [37]. This proves the ability of NIR to determine a broad range of CQAs and even some CPPs, which allows it to be an all-in-one monitoring technique.

A disadvantage of NIR is the interference created by water when analyzing aqueous solutions. The O-H bond of water are detected and lie directly over the other bonds of interest. These dominant water peaks will be present at approximately 1450 and 1940 nm. Research is done in how to overcome these absorption features in order to study the amount of carbo-hydrates, protein and lipids [16].

VI. Recent research

For a complete overview of all research discussed, see Appendix 1 [28].

A. *Off-line and At-line*

The use of off-line monitoring of nutritional values of algae has been researched several times.

Corresponding with this research, Laurens et al. [27] successfully predicted the amount of carbo-hydrates, protein and lipids in freeze-dried biomass of three strains of algae. The study gathered this data by using a calibration model, which only looked at specific parts of the NIR spectrum, wavelength 1725 and 2305 nm. The amount of lipids of other species could be determined with a correlation of $R^2 = 0.86$ and 0.77, respectively. However, this study found that recalibration of wavelengths is needed in order to correctly quantify the amount of carbohydrates and protein. The correlation between these was 0.38 and 0.76 with large scatter. In addition, the wavelength 1215 nm was investigated, but found unsatisfactory.

Liu et al. [29] also found a successful way of determining fatty acid methyl esters (FAMEs), needed for biodiesel using NIR. By scanning the lyophilized cells, the amount of FAMEs was gained within seconds with a mean correlation greater than 0.99.

Brown et al. [30] found that NIR could be used on still moist algae, who have just been filtered for 1 minute by glass-fiber filters [30]. They also determined the amount of FAMEs, and found correlations of 0.96 and 0.89 for two different species. The used technique can be used in an at-line monitoring procedure, allowing for faster feedback to the cultivation process than the freeze-dry technique.

Ge et al. [26] investigated the possibility to use NIR in lyophilized algae cells in order to determine the amount of neutral lipids, protein and gross calorific value (GCV). PLS was used to calibrate the model that relates the absorption spectrum and the chemical data from the lab. The model was able to predict the amount of protein with $R^2 = 0.90$, lipids with $R^2 = 0.70$ and the GCV with $R^2 = 0.57$.

This research discussed that the application of this technology to algae culture with biomass around 0.1% would be challenging. However, a proposal is quoted which suggests that NIR and Raman spectrometry can complement each other in order to solve the problem with water interfering with the nondestructive analysis of algae components [31, 32].

B. *On-line and In-line*

There are successful studies done on implementing NIR in order to determine biomass. Meireles et al. [34] made an automated on-line closed feedback loop by using NIR as monitor of biomass to control the density of the algae culture by automatic injection of growth medium. However, the use of NIR as an in-line monitor of nutritional values has yet to be fully utilized. As of now, some studies have tried to implement NIR in-situ for this purpose, but the results have been equivocal.

Shoa et al. [18] have successfully used NIR in-situ to predict the amount of carotenoid content. The amount of carotenoids were predicted with a correlation of 0.96 by using the wavelengths of 475.92 and 506.58 nm. Although these wavelengths lie in the visual light region, another effective wavelength was found at 840 nm, proving the possibility of the NIR region to predict the amount of carotenoids. This wavelength had to be disregarded however, because it could not be explained yet. They used three different calibration methods, all variations on PLS.

Zhou et al. [35] found the possibility of using an immersed VIS/NIR spectrometer for rapid detection of chlorophyll a and b. They found RPD values of 2.9461 and 1.9023 for chlorophyll a and b. This indicates that their PLS calibration model combined with their chosen spectrum can predict chlorophyll amount in algae.

Challagulla et al. [23] have researched the possibility to use NIR in-situ (in flask) to determine the amount of biomass and lipids. They found a correlation of 0.85 to biomass, but their value for lipids was not satisficing (R^2 = -0.18). A recommendation is given that this instrument technique is not capable of assessment of lipid content in-situ.

The potential however is still forthcoming due to its successful use in other bioprocesses. Several review articles prove the capability of NIR in on-line/in-line application [13, 20, 21]. These articles mention the advances NIR has made in the food, bio-based, pharmaceutical and biomedical industry. Its usability to in-line monitor the amount of protein and fat in milk [22], the amount of glucose in mammalian cells [24] or in fermentation processes have been proven [25]. Its development in the pharmaceutical and biomedical field provide the desire for progressing this technology to higher standards [38, 39]. The widespread application of the technology gives reason to belief it is possible to advance it to a stage where it can predicts the amount of carbo-hydrates, proteins and lipids in-situ.

VII. RECOMMENDED RESEARCH

Other spectrometers are recommended in some of the literature referenced to in this review. Most notable are Raman spectrometry and Fourier-transformed attenuated total reflection infrared spectrometry (FT-ATR).

Raman spectrometry is a relatively new technique, which is the reason why it has not been tested widely in the algae cultivation field. However, the vast majority of the studies done focus on the analysis of *in situ* algae. This can be done because Raman spectrometry receives weak spectral interference from water, making it a desirable tool for analyzing biological samples. The downside to this technique is the disturbance created by background fluorescence spectra, which makes this technique less qualified to test algae with highly fluorescent pigments. Despite this shortcoming, Raman spectrometry successfully predicted *in-line* the amount of glucose, lipids, carbohydrates, protein, carotenoids and biomass [11].

FT-ATR is also a relative new tool, but proven to have great potential as a monitor of CQAs of bioprocesses. This technique uses the mid infrared spectrum, which contains the fundamental vibration absorption wavelengths of the molecular bonds of organic compounds. To prevent interference from water, FT-ATR only penetrates the adjacent medium in the order of micrometers. This small effective path length allows spectra to be obtained even in the water bands. In combination with Fourier transformation analyses, spectra of aqueous-phase biomolecules can be obtained in the entire mid-IR range [40]. Because it is a new technique, it has not seen extensive use in algae monitoring, but it has been used to predict the amount of glucose *in-line* successfully [41].

VIII. DISCUSSION

Over the last decade, NIR has seen extensive usage in algae industry as an on-line turbidimetric *optical density* tool to estimate microalgae density. This has made it possible to build closed loop systems for optimal algae cultivation. However, the nutritional values of the product of these systems were not monitored.

Studies have successfully tried to use NIR *on-line in situ* to monitor values like carotenoids and chlorophylls in algae. These studies were effective because of good calibration methods and wavelength selection. One study tried to monitor lipid value, but was unsuccessful.

Other studies evaluated the possibility of using NIR *off-line* and found a favorable outcome for predicting the amount of lipids and protein. One study has tried to evaluate the amount of carbo-hydrates, but was unsuccessful in using the researched wavelength to predict carbo-hydrates in unknown species.

Unfortunately, to this date, the nutritional values of algae have not been elucidated by NIR technology *in-line*. This is largely because the algae are grown in a low ratio to water (around 0.1%). The interference caused by this is overshadowing the values of the nutrients.

Research is needed to determine which specific wavelengths translate to specific nutritional values. In addition, NIR spectrometers have to become more precise in order to distinct which reflections are caused by water bonds and which are caused by larger molecules. Furthermore, to translate these detailed values to nutritional values, the spectrum-substance correspondence as seen in Figure 3 has to become more in depth and specific. Once these recommendations have been met, the possibility to monitor algae *in-line* with NIR technology can be explored. However, the possibility of using it *at-line* has a good chance of success. Although specific wavelengths to monitor the amount of carbo-hydrates in algae have not yet been found, no study has ever had this as its main goal either. If found, and found compatible with the wet filtrate technique proposed by Brown et al. [13], a quick at-line monitoring procedure becomes feasible. Although this is less desirable over an in-line monitor, it would allow NIR to be used commercially, generating more demand for research in the technique. This could be the launch it needs to develop NIR in a technology capable of scanning at the substance water ratio that is needed to monitor algae cultivation.

IX. CONCLUSION

For now, this means that NIR technology still has a long way to go. It is not capable enough to monitor the CQAs of the algae cultivation process. This renders the technology not qualified to monitor industry standards for nutritional values *in-line* as recommended by European and American institutes. Nevertheless, the nutritional values of algae can be monitored by NIR in a later stage than cultivation. If scanned in a better biomass to water ratio, NIR is capable of determining the algae composition. This could be a practical solution to the interference problem, with as only downside that miss-batches can occur. This would allow the development of an algae based food supplement product that meets medical standards of quality. Consequently, the patients requiring could enjoy all the beneficial properties of algae.

X. ACKNOWLEDGMENT

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XI. REFERENCES

[1] Nielsen Global Health and Ingredient-Sentiment Survey 2016

[2] Manual of clinical nutrition management 2013

[3] Cockbain AJ, Toogood GJ, Hull MA: Omega-3 polyunsaturated fatty acids for the treatment and prevention of colorectal cancer: *Gut* 2012;61**:**135-149.

[4] Kay, R. A., & Barton, L. L. (1991). Microalgae as food and supplement. Critical Reviews in Food Science and Nutrition, 30(6), 555–573.doi:10.1080/10408399109527556

[5] Fidalgo, J. (1998). Effects of nitrogen source and growth phase on proximate biochemical composition, lipid classes and fatty acid profile of the marine microalga Isochrysis galbana. Aquaculture, 166(1-2), 105-116.

[6] REICH, G. "Near-Infrared Spectroscopy And Imaging: Basic Principles And Pharmaceutical Applications". *Advanced Drug Delivery Reviews*, vol 57, no. 8, 2005, pp. 1109-1143. *Elsevier BV*, doi:10.1016/j.addr.2005.01.020.

[7] Pasquini, Celio. "Near Infrared Spectroscopy: Fundamentals, Practical Aspects And Analytical Applications". *Journal Of The Brazilian Chemical Society*, vol 14, no. 2, 2003, pp. 198-219. *Fapunifesp (Scielo)*, doi:10.1590/s0103-50532003000200006.

[8] DU, Yi Ping et al. "Removal Of Interference Signals Due To Water From In Vivo Near-Infrared (NIR) Spectra Of Blood Glucose By Region Orthogonal Signal Correction (ROSC)". *Analytical Sciences*, vol 20, no. 9, 2004, pp. 1339-1345. *Japan Society For Analytical Chemistry*, doi:10.2116/analsci.20.1339.

[9] European Federation of Biotechnology. European Federation of Biotechnology Section on Biochemical Engineering Science (ESBES) [Internet]. 2016. Available from: http://www.efb-central.org/index.php/Main/section_on_biochemical_engineering_science.

[10] U.S. Department of Health and Human Services Food and Drug Administration. Guidance for Industry – PAT – a Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance. 2004.

[11] Podevin, M., Fotidis, I. A., & Angelidaki, I. (2017). Microalgal process-monitoring based on high-selectivity spectroscopy tools: status and future perspectives. Critical Reviews in Biotechnology, 38(5), 704–718.doi:10.1080/07388551.2017.1398132

[12] ICH. Pharmaceutical Development Q8(R2): ICH Harmonised Tripartite Guideline. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use [Internet]. 2009 [cited 2016 Dec 3]. p. 1–24. Available from: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/ Q8_R1/Step4/Q8_R2_Guideline.pdf.

[13] Zhao, L., Fu, H., Zhou, W. and Hu, W. (2015). Advances in process monitoring tools for cell culture bioprocesses. *Engineering in Life Sciences*, 15(5), pp.459-468.

[14] Thermo fisher scientific – NIR technology, accessed at 26-4-2019 https://www.thermofisher.com/nl/en/home/industrial/spectroscopy-elemental-isotope-analysis/spectroscopy-elemental-isotope-analysis-learning-center/molecular-spectroscopy-information/nir-technology.html

[15] Wu, Z., Ouyang, G., Shi, X., Ma, Q., Wan, G. and Qiao, Y. (2014). Absorption and Quantitative Characteristics of C-H Bond and O-H Bond of NIR. *Оптика и спектроскопия*, 117(5), pp.724-730.

[16] OZAKI, Y. (2012). Near-Infrared Spectroscopy; Its Versatility in Analytical Chemistry. *Analytical Sciences*, 28(6), pp.545-563.

[17] Sandnes JM, Ringstad T, Wenner D, et al. Real-time monitoring and automatic density control of largescale microalgal cultures using near infrared (NIR) optical density sensors. J Biotechnol. 2006;122: 209–215.

[18] Shao Y, Pan J, Zhang C, et al. Detection in situ of carotenoid in microalgae by transmission spectroscopy. Comput Electron Agric. 2015;112:121–127.

[19] Challagulla V, Nayar S, Walsh K, et al. Advances in techniques for assessment of microalgal lipids. Crit Rev Biotechnol. 2017;37:566–578.

[20] Landgrebe D, Haake C, H€opfner T, et al. On-line infrared spectroscopy for bioprocess monitoring. Appl Microbiol Biotechnol. 2010;88:11–22.

[21] Lourenc¸o ND, Lopes JA, Almeida CF, et al. Bioreactor monitoring with spectroscopy and chemometrics: a review. Anal Bioanal Chem. 2012;404:1211–1237.

[22] Laporte MF, Paquin P (1999) Near-infrared analysis of fat, protein, and casein in cow's milk. J Agric Food Chem 47:2600–2605

[23] Challagulla V, Walsh KB, Subedi P. Biomass and total lipid content assessment of microalgal cultures using near and short wave infrared spectroscopy. Bioenerg Res. 2014;7:306–318.

[24] Arnold SA, Crowley J, Woods N, Harvey LM, McNeill B (2003) In-situ near infrared spectroscopy to monitor key analytes in mammalian cell cultivation. Biotechnol Bioeng 84:13–19

[25] Tosi S, Rossi M, Tamburini E, Vaccari G, Amaretti A, Matteuzzi D (2003) Assessment of in-line near-infrared spectroscopy for continuous monitoring of fermentation processes. Biotechnol Prog 19:1816–1821

[26] Ge Y. NIR reflectance and MIR attenuated total reflectance spectroscopy for characterizing algal biomass composition. Trans ASABE. 2016;59:435–442.

[27] Laurens LML, Wolfrum EJ. High-throughput quantitative biochemical characterization of algal biomass by NIR spectroscopy; multiple linear regression and multivariate linear regression analysis. J Agric Food Chem. 2013;61:12307–12314.

[28] Liu, J.-Y., Zeng, L.-H., & Ren, Z.-H. (2019). Recent application of spectroscopy for the detection of microalgae life information: A review. Applied Spectroscopy Reviews, 1–34. doi:10.1080/05704928.2018.1509345

[29] Liu, B., Liu, J., Chen, T., Yang, B., Jiang, Y., Wei, D., and Chen, F. (2015) Rapid characterization of fatty acids in oleaginous microalgae by near-infrared spectroscopy. Int. J. Mol. Sci. 16(4): 7045–7056.

[30] Brown, M. R., Frampton, D. M. F., Dunstan, G. A., and Blackburn, S. I. (2014) Assessing near-infrared reflectance spectroscopy for the rapid detection of lipid and biomass in microalgae cultures. J. Appl. Phycol. 26(1): 191–198.

[31] Samek, O., Zemanek, P., Jonas, A., and Telle, H.-H. (2011) Characterization of oil-producing microalgae using Raman spectroscopy. Laser Phys. Lett. 8(10): 701–709.

[32] Huang, Y. Y., Beal, C. M., Cai, W. W., Ruoff, R. S., and Terentjev, E. M. (2009) MicroRaman spectroscopy of algae: Composition analysis and fluorescence background behavior. Biotech. Bioeng., 105(5), 889–898

[33] Brian G. Osborne (2006) Near-infrared Spectroscopy in Food Analysis. BRI Australia Ltd, North Ryde, Australia

[34] L.A. Meireles, J.L. Azevedo, J.P. Cunha, F. Xavier Malcata On-line determination of biomass in a microalgal bioreactor using a novel computerized flow injection analysis system Biotechnol. Prog., 18 (6) (2002), pp. 1387-1391

[35] Zhou, H., Fang, H., Pan, J., Jiang, L., He, Y., and Shao, Y. (2017) Chlorophyll contentresearch of Haematococcus pluvialis based on immersed visible/near-infrared spectroscopy. Spectrosc. Spect. Anal. 37(11): 3375–3378.

[36] Robert Mattes Denise Root Misa A. Sugui Fan Chen Xiao Shi Jonathan Liu Philippe-Alexandre Gilbert (2009) Real-Time Bioreactor Monitoring of Osmolality and pH Using Near-Infrared Spectroscopy. Bioprocess International

[37] Sandor, M., Rüdinger, F., Solle, D., Bienert, R., Grimm, C., Groß, S. and Scheper, T. (2013). NIR-spectroscopy for bioprocess monitoring & control. BMC Proceedings, 7(Suppl 6), p.P29.

[38] Jamrógiewicz, M. (2012). Application of the near-infrared spectroscopy in the pharmaceutical technology. Journal of Pharmaceutical and Biomedical Analysis, 66, pp.1-10.

[39] Rani, Asha & Singh, Vijander & Murari, Bhaskar. (2013). NEAR-INFRARED SPECTROSCOPY IN BIOMEDICAL APPLICATIONS. Unpublished

[40] Suci, P. A., Vrany, J. D., & Mittelman, M. W. (1998). Investigation of interactions between antimicrobial agents and bacterial biofilms using attenuated total reflection Fourier transform infrared spectroscopy. Biomaterials, 19(4-5), 327–339. doi:10.1016/s0142-9612(97)00108-7

[41] Girard, J., Deschênes, J., Tremblay, R. and Gagnon, J. (2013). FT-IR/ATR univariate and multivariate calibration models for in situ monitoring of sugars in complex microalgal culture media. Bioresource Technology, 144, pp.664-668.

[42] Barbin, D., Felicio, A., Sun, D., Nixdorf, S. and Hirooka, E. (2014). Application of infrared spectral techniques on quality and compositional attributes of coffee: An overview. Food Research International, 61, pp.23-32.

Appendix 1. Applications of NIR spectrometry for the determination of microalgae life information

Species	Analyte	Spectral range (nm)	Method	Evaluation parameters	Advantages	Limitations
	Ash content	1000–2500 nm	PLSR	r^2=0.53; RPD =1.48		
Chlorella vulgaris	Biomass	300–1100 nm 1100-2500 nm	MSC-PLSR	R_{CV}=0.955 SDR =3.38	A low-effort sample preparation method (dry filtrates)	The robustness of the PLSR models across growth conditions
	Lipid	300–1100 nm 1100-2500 nm	MSC-PLSR	R_{CV}=0.874 SDR =2.06		
Chlorella sp.	Lipid content	400 – 2500 nm	MSC-SNV-PLS	R^2_c=0.91 RMSEC =3.84		
	Carbohydrate	400 – 2500 nm	MSC-SNV-PLS	R^2_c=0.83 RMSEC =5.31		
	Protein content	400 – 2500 nm	MSC-SNV-PLS	R^2_c=0.96 RMSEC =2.11	The use of NIR for the full biochemical composition of microalgal biomass, using a combined species prediction model	Lack of cross-species prediction across more widely different algal species
Scenedesmus sp.	Lipid content	400 – 2500 nm	MSC-SNV-PLS	R^2c = 0.91 RMSEC =3.84		
	Carbohydrate	400 – 2500 nm	MSC-SNV-PLS	R^2c = 0.91 RMSEC =3.84		
	Protein content	400 – 2500 nm	MSC-SNV-PLS	R^2c = 0.91 RMSEC =3.84		
Nannochloropsis sp.	Lipid content	400 – 2500 nm	MSC-SNV-PLS	R^2c = 0.91 RMSEC =3.84		
	Carbohydrate	400 – 2500 nm	MSC-SNV-PLS	R^2c = 0.91 RMSEC =3.84		
	Protein content	400 – 2500 nm	MSC-SNV-PLS	R^2c = 0.91 RMSEC =3.84		
Kirchneriella sp.	Biomass	1110–1900 nm	MSC-PLS	R^2=0.96 RPD =4.8	Rapid screening	For Nannochloropsis sp., the model metrics were less favorable
	Fatty acid methylesters (FAME)	1110–1900 nm	MSC-PLS	R^2=0.97 RPD =5.6		
Nannochloropsis sp.	Biomass	1110–1900 nm	MSC-PLS	R^2=0.85 RPD =2.6		
	Fatty acid methylesters (FAME)	1110–1900 nm	MSC-PLS	R^2=0.84 RPD =2.5		
Pluvialis flotow	Chlorophyll a	346–1038 nm	SG-PLS	RPD =2.6925	Two spectral preprocessing Algorithms of SG and SNV are synthetically analyzed	The reference light is not calibrated, which will increase the measurement error.
	Chlorophyll b	346–1038 nm	RAW-PLS	RPD =1.9477		
Spirulina sp.	Carotenoids	346–1038 nm	SPA-PLS	RPD =3.44	*In situ* measurement of carotenoids	There was a lot of noise at both terminals of the spectra curve
Nannochloropsis sp.	Triglyceride	400–2500 nm	MSC-no Vis-PLS	RMSECV =0.182 R^2=0.969	They verify the accuracy of prediction the combined multiple-species models	Did not consider the impact of water
	Phospholipid	400–2500 nm	MSC-no Vis-PLS	RMSECV =0.227 R^2=0.951		
Chlorella sp.	Lipids	325–1075 nm	SPA-MLR	RPD =1.7537	Spectral measurements under different light condition	Did not consider the impact of water
Chlorella sp.	Fatty acids	1030–1500 nm 1600–1880 nm	PLS	RPD =4.98	Quantification of individual fatty acids in microalgae	Other fatty acids in trace amounts were not considered
	Palmitic acid	1030–1500 nm 1600–1880 nm	PLS	RPD =3.58		
	Stearic acid	1030–1500 nm 1600–1880 nm	PLS	RPD =3.29		
	Oleic acid	1030–1500 nm 1600–1880 nm	PLS	RPD =4.81		
	Linoleic acid	1030–1500 nm 1600–1880 nm	PLS	RPD =2.92		
	Linolenic acid	1030–1500 nm 1600–1880 nm	PLS	RPD =4.80		
Algal turf scrubber	Ash content	1100–2500 nm	PLSR	r^2=0.994	Require only one spectrum, available in minutes at most, real-time measurements	Can not accurately determine lipid or total fatty acid content in ATS samples
	N	1100–2500 nm	PLSR	r^2=0.787		
Nannochloropsis and Nannochloris	Neutral lipids	1000–2500 nm	PLSR	r^2=0.77; RPD =2.1	Eliminate the influence of water	High ash content, Neutral lipid value significantly lower compared to literature values
	Crude protein	1000–2500 nm	PLSR	r^2=0.77; RPD =2.1		
	Gross calorific	1000–2500 nm	PLSR	r^2=0.77; RPD =2.12		