Interreg
North-West Europe
ALG-AD
European Regional Development Fund

EUROPEAN UNION

THEMATIC PRIORITY

RESOURCE AND MATERIAL EFFICIENCY

BEST PRACTICES GUIDELINE FOR CULTIVATION OF MICROALGAE ON NUTRIENT RICH DIGESTATE

PART 1 :

BEST PRACTICES FOR THE TREATMENT AND PREPARATION OF NUTRIENT RICH DIGESTATE FOR ALGAL CULTIVATION

EDITION : MARCH 2020

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Acknowledgements

The authors would like to thank **Louise Hall, Catharine Jones and Clara Diebolt** for proofreading and formatting this document; **Eleanor Wood** and **Vanessa Ndovela** for contributing to the experimental work and proof reading; **Mathilde Castagnet, Robert Lovitt, Tom Chaloner** and **Lynsey Melville** for their expertise and advice.

Please cite this document as follow:

Fernandes F.*, Aouamri N., Fernandes de Souza M., Fuentes-Grünewald C., Jones G., Seelam J.S., Sigurnjak I., Llewellyn C.A.* 2021. Best Practices for the treatment and preparation of nutrient rich digestate for algal cultivation. Public Output report of the ALG-AD project, Swansea, June 2021, 47 pages.

Available online at:
www.nweurope.eu/projects/ALG-AD

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This document is an output from the ALG-AD project, which has received European Regional Development Funding through the INTERREG IVB NWE programme.

Executive summary

This document is a compilation of Best Practices recommendations and considerations for the preparation of nutrient rich digestate (NRD) with the purpose to be used as a nutrient source in microalgae cultivation systems. This document is part of the INTERREG North West Europe funded ALG-AD project aiming to combine anaerobic digestion (AD) and algal cultivation technologies to remediate nutrient rich digestate currently produced in excess in the region.

In this report, best practices are presented for preparing NRD, including health and safety procedures, characterisation of the digestate with an emphasis on key parameters and recommendations for analysis, treatment of the digestate to facilitate integration into microalgal cultivation systems and reach optimal nutrient concentrations for microalgal growth. The document draws on experiments and trials conducted on digestate provided by three anaerobic digestion plants in the United Kingdom, Belgium and France and additional findings can be found in Fernandes et al., 2020

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Glossary

AD: Anaerobic Digestion

NRD: Nutrient Rich Digestate

OECD: Organisation for Economic Co-operation and Development

PAS110: Publicly Available Specification 110

QP: Quality Protocol

WRAP: Waste and Resources Action Programme

IBC: Intermediate Bulk Container

PPE: Personal Protective Equipment

ICP-OES/ ICP-AES: Inductively Coupled Plasma Emission Spectrometry / Inductively Coupled Plasma-Atomic Emission Spectrometry

XRF: X-ray Fluorescence

TAN: Total Ammoniacal Nitrogen

VFAs: Volatile Fatty Acids

RCF: Relative Centrifugal Force

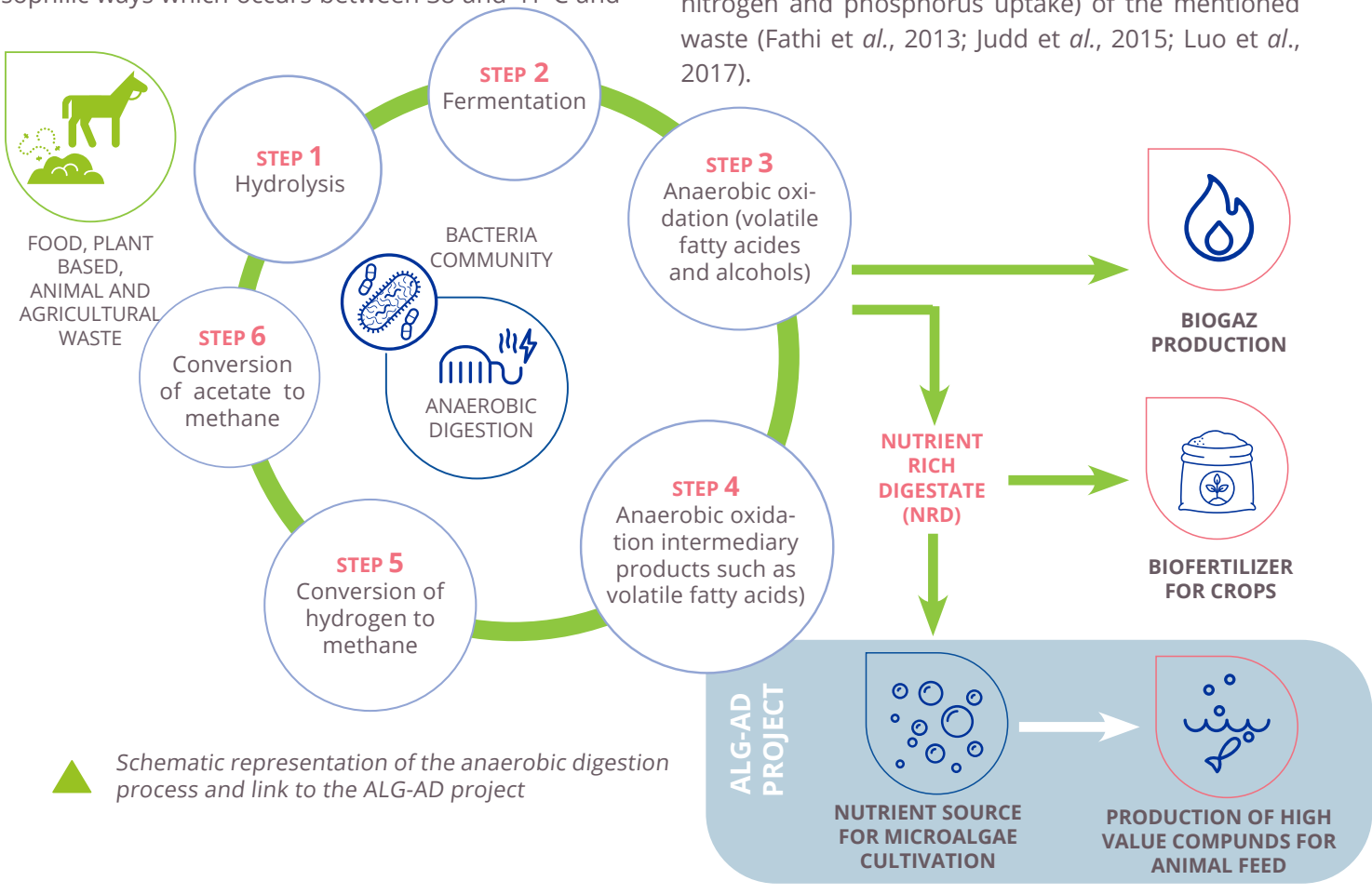
DAF: Dissolved Air Flotation

1 INTRODUCTION

Anaerobic digestion of waste

With the increase of global industrialisation, a considerable amount of waste is being generated. Consequently, it is necessary to find solutions to treat this waste in order to limit anthropogenic impact on natural ecosystems and environments. Waste can be biologically treated to produce biogas that is reused for energy purposes: this is the anaerobic digestion process that is a technology widely used for the treatment of carbon-rich organic waste. Anaerobic digestion (AD) is a biological process during which organic matter (e.g. food, animal or agricultural waste) is converted by bacterial and archaeal communities cooperating to form a stable and self-regulating fermentation process that transforms the organic matter into biogases primarily composed of methane (CH_4) and carbon dioxide (CO_2) (Doble and Kumar, 2005). This process occurs in a tank called digester and can be conducted under different temperatures : the mesophilic ways which occurs between 38 and 41°C and the thermophilic way operated at 52°C. The volume of biogas produced is dependent on the type of waste and its composition, as well as the quantity of waste treated and the operating temperature of the digester. Anaerobic digestion can be divided into six stages (Jeyaseelan, 1997): hydrolysis of complex organic biopolymers into monomers; fermentation of amino acids and sugars; anaerobic oxidation of volatile fatty acids and alcohols; anaerobic oxidation of intermediary products such as volatile fatty acids; conversion of hydrogen to methane; conversion of acetate to methane. Commonly the AD process is divided into hydrolysis, acidogenosis, acetogenosis and methanogenosis stages. This process results in the production of biogas converted into electricity by a co-generator or directly used by injection after purification. The AD process also results in the production of a nutrient rich liquid digestate with a high dry matter content (Figure 1).

Digestate is often extremely rich in carbon, nitrogen, phosphorus and other macro and micronutrients (Papadimitriou et *al.,* 2008; Tambone et *al*., 2017). All of these components are key factors in the growth and development of microalgae, which are aquatic photosynthetic, mixotrophic and/or heterotrophic microorganisms. When grown on wastewater or liquid digestate as a medium substrate, microalgae present a significant potential for the bioremediation (e.g. nitrogen and phosphorus uptake) of the mentioned waste (Fathi et *al.*, 2013; Judd et *al.*, 2015; Luo et *al.*, 2017).



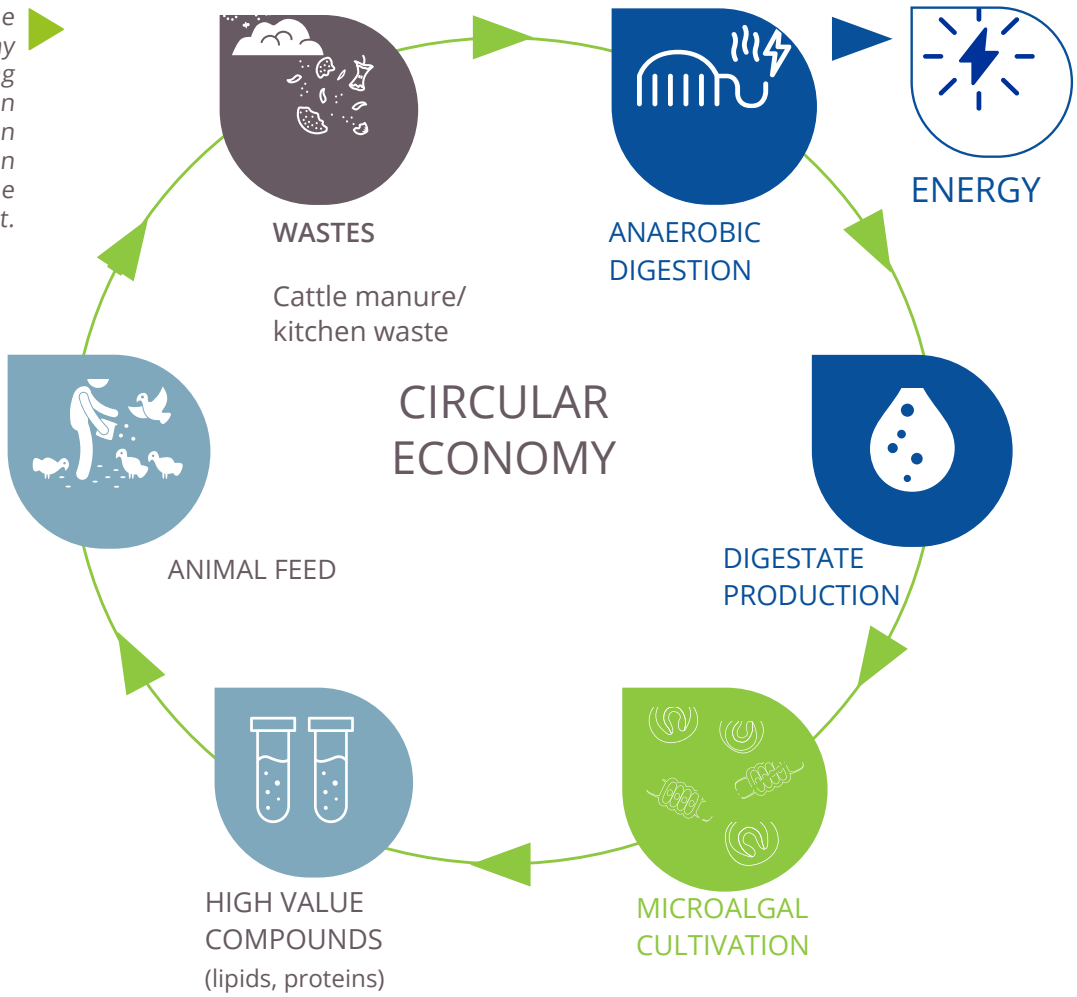
Schematic representation of the anaerobic digestion process and link to the ALG-AD project

The ALG-AD Project

Langage AD pilot site

The ALG-AD project (INTERREG-NWE funded - www.nweurope.eu/projects/ALG-AD) addresses reuse of waste to generate products for a sustainable economy, reducing pollution risk and dependence on imported material resources. The project focuses on the use of nutrient rich digestate, produced extensively in North West Europe (NWE), which is an important agricultural region considerably impacted by the eutrophication of soils. Growing microalgae on this substrate will significantly reduce the nutrient concentration in the digestate and allow the use of excess nutrient rich digestate in a new commercial area, enabling application of a circular economy approach. Furthermore, the algal biomass produced would be a valuable resource, potentially fed back into the production chain as an animal feed or for other industrial purposes (e.g. plant biostimulants, cosmetic additives) due to the microalgae's high content in valuable compounds and metabolites such as proteins, lipids, and vitamins. This will allow the creation of a circular and sustainable economy with a very limited generation of wastes (Figure 2). Further details on the ALG-AD project can be found in Appendix 1.

Schematic of the circular economy principle combining anaerobic digestion and algal cultivation technologies within the framework of the ALG-AD project.



The combination of AD and algal technologies will be collaboratively implemented and tested in three distinct 'real-life conditions' pilots in Devon (UK), Brittany (FR) and Flanders (BE). Sites reflect the heterogeneity of NWE from 'predominantly rural remote' to 'predominantly urban' (OECD 2011) and different types of biodegradable waste in different regulatory landscapes.

Scope of the Best Practice Document

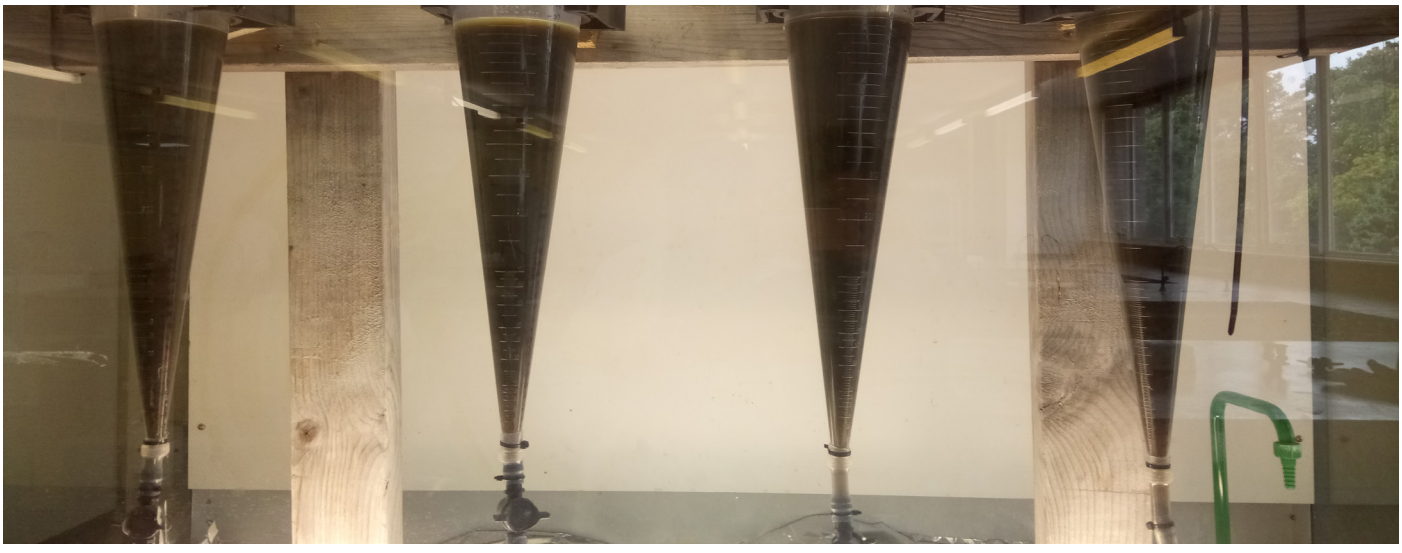
A nutrient rich digestate appears as an ideal and cost effective substrate for the cultivation of microalgae (Olguín et al., 2012; Craggs et al., 2013). However, digestate needs to be analysed in depth before its utilisation in microalgae cultures. Indeed, it is necessary to know the exact composition of digestate used for algal cultivation, especially in terms of nutrient concentration, to ensure efficient growth in culture (Xia and Murphy, 2016). Hence, it is crucial to use an optimised digestate at an adequate concentration. Furthermore, the quality of algal growth will depend on the digestate used and its provenance as well as how it has been treated prior to its use as a feedstock for microalgal cultivation (i.e. upstream process in microalgal cultivation).

The aims of this document are to describe and recommend best practices for the general handling and use of nutrient rich digestate; its characterisation (and associated analytical methods) and its preparation and treatment for large scale microalgal cultivation. This document draws on experimental protocols and results gathered from the different academic partners and investments of the ALG-AD project (Table 1). Furthermore, detailed protocols and information on the operational sites are gathered in Appendix 2 (1&2). Results are shown throughout the document as analysed by Swansea University (SU) and Innolab.

Liquid digestate after separation of phase

Brief description of AD plants collaborating on the ALG-AD project and their digestate production per year.

AD Plant Name	Country	Digestate sourcing	Digestate production yield	Detailed description
Langage AD	United Kingdom (Devon)	Food waste	20 000 t/y	Appendix 2
Cooperl Arc Atlantique	France (Brittany)	Pig manure		Appendix 2
Innolab outsourced AD-plant	Belgium (Flanders)	Food and plant waste	160 000 t/y	



2 HEALTH AND SAFETY CONSIDERATIONS FOR DIGESTATE UTILISATION

Risks associated with the utilisation of digestate

Anaerobic digestion is a specific process involving numerous risks linked to the potential exposure of individuals to flammable atmospheres, pressurised systems and harmful gases and chemicals. Furthermore, there is a risk associated with the **microorganisms' populations** (potential pathogens) present in the digesters, which are necessary for the anaerobic digestion process. While anaerobic digestion facilities have risk assessments and health and safety protocols in place to carry out the anaerobic digestion process and store the resulting digestate, using digestate for microalgal growth is a new concept that could involve new risks needing to be addressed to meet **health and safety requirements** in the workplace.

The risks associated with using digestate for microalgal growth are directly correlated with the **volume used**. Indeed a higher volume of digestate is more likely to cause harm to individuals and to the environment. Toxic **gases**, such as methane (CH_4) or hydrogen sulphide (H_2S) are likely to form during transport or storage of digestate and **spillage** in the environment could occur causing toxicity to impacted wildlife. Furthermore, there is a risk associated with **microorganisms**. While their concentration is significantly reduced by high temperature treatments or pasteurisation during the AD process, they can redevelop if digestate is stored in poor environmental conditions (e.g. room temperature). These microorganisms and pathogens are potentially harmful to individuals and can cause infections.

Certifications scheme

In order to assess and control the aforementioned risks, especially regarding pathogens, certifications have been put in place to assess the **quality of digestates** produced and used as biofertilisers. In the UK, the **biofertilisers certification scheme** is the most common certification of digestates and is part of the Renewable Energy Assurance Limited group of assurance scheme. This organisation has been created to certify biogas/AD plants against the Publicly Available Specification 110 (PAS110) and the associated quality protocol (QP). This ensures that "digested materials are made using suitable inputs and effectively processed by AD for a sufficient time, ensuring that the process has been well managed and monitored to produce digested material that meets market needs and protects the environment" (WRAP, BSI, 2010).

In order to obtain these certifications, the digestates produced have to meet criteria required to pass a **quality protocol.** Compliance with this protocol is sufficient to ensure that the digestate resulting from AD can be used. The digestate produced by the AD facility **LANGAGE AD** (Devon, UK) and sourced from kitchen waste is **certified PAS 110** by the biofertilisers certification scheme and consequently does not require any permit to be used. Following this certification, the digestate produced by LANGAGE AD should be treated as a microbiological hazard of **category 1**, meaning that the microorganisms potentially present in this digestate are unlikely to cause disease. The digestate produced by **Cooperl Arc Atlantique** (Brittany-France) is **certified ISO 9001-2015** (FR 22 261 004).

The Waste and Resources Action programme (WRAP) investigated the safety of digestate meeting the PAS 110 quality specification, when used on land as a biofertilisers. Microbiological, physical and chemical risks were considered. Different risk scenarios showed that the risk from digestate were **very low or negligible**, demonstrating that digestate certified PAS 110 was safe to be used on land. While there is **no clear regulation** for the use of digestate for microalgal cultivation yet, this report confirms the potential of using digestate for this specific area (providing that the microalgae and high value compounds produced are used in an adequate field). In other words, **waste-derived digestate and compost become products** (i.e. they are no longer wastes) once certified under the relevant scheme.

A detailed microbiological analysis of digestate from the three investment sites has been completed, and results will be made available in a specific Safety Analysis report

Handling and storing the digestate

Transport

Transport of digestate should be carefully considered according to the **volume** transported and **means** put in place to carry out the transport in a safe manner. The **delivery time** should be minimised to avoid degradation of the digestate and (when possible) the digestate should be maintained at a **low temperature (e.g. refrigerated transport)**. The digestate can be transported in **plastic containers** (according to the volume, this could be IBC, canisters, barrels, etc.). However, regardless of the container used, a secondary containment should be put in place (e.g. bund, spill tray) to contain any potential spill in the vehicle used for transport or in the environment. Furthermore, the container used should always be filled to no more than three quarters of its total capacity. Indeed, in case of biogas formation during transport (e.g. methane and H_2S), there would be enough free headspace for the gas to be contained, avoiding pressure formation in the container.

Storage requirements

The digestate should be stored at a low temperature (i.e. 4-8°C) to avoid development of microorganisms and to avoid any changes in its initial composition, as well as any release of gases (such as ammonia gases) (Olguin et al., 2015; Tao et al., 2017; Wahal and Viamajala, 2016). Indeed, digestate is a product that has the potential to carry on maturing after being collected from the digester. There should be a second containment put in place when storing digestate (spill tray for small containers and bund for IBC) in order to contain any potential spill linked to damaged containers. The containers used (canisters, barrels, IBC) should be able to block light to avoid degradation of the digestate due to high light intensity. Furthermore, pasteurisation could also be envisaged when storing digestate, as this treatment would remove any biological contamination without affecting the digestate composition.

Containers with liquid digestate used at Innolab pilot site

Handling

When handling the digestate for processing or use for microalgal cultivation, standard personal protective equipment (PPE) should be worn at all times. Lab coat, safety glasses, face mask and gloves should be worn for any kind of activity involving the use of digestate in order to avoid spillage onto skin, clothes or in the eyes. Users should be working in a well-ventilated location in case of biogas formation, but if working in a confined space, respiratory protection should be used in order to avoid inhalation of harmful gases that could have formed during the digestate transport or storage. When working with a high volume of digestate, for example moving or displacing the digestate, protective footwear (e.g. rubber boots) should be worn to avoid contact with skin or clothes in case of spillage.

Risk assessments and accidental release scenario

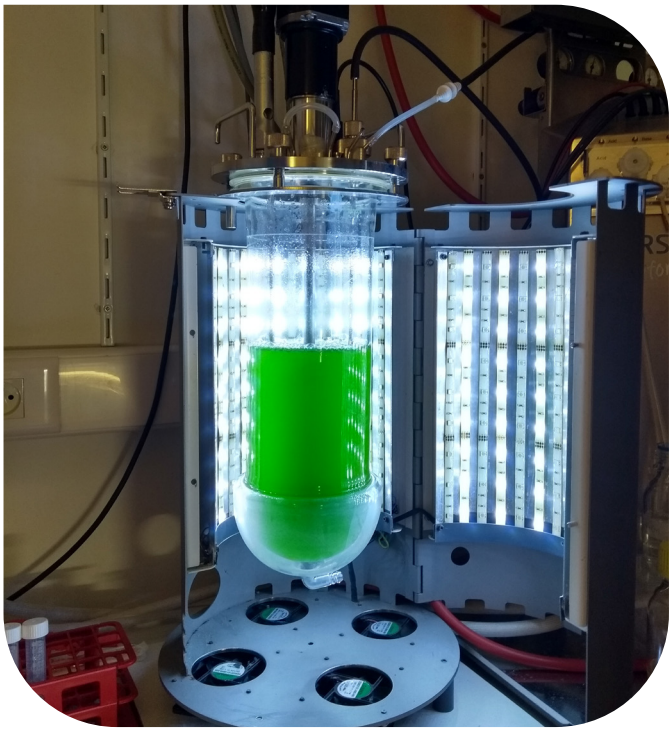
Upon using digestate for processing purposes, microalgal cultivation or other uses, a full risk assessment should be undertaken tackling the different topics discussed above. The risk assessment must consider the volume of digestate used, as this will affect the hazard level and the likelihood of accidental release occurring (the higher the volume, the higher the risk). The risk assessment should also include all types of treatment procedure used to handle and process the digestate, such as thermic treatment or procedures involving chemicals as they may inhibit some properties of the digestate and increase its hazard.

In case of accidental release or a spill on an individual, measures should be taken to limit the spread of the spill. For example, a spill kit should be available in order to contain any spill. In case of accidental release or spill on individual, remove the clothes in contact with the digestate and rinse the skin abundantly if it was in contact with the digestate. In case of eye exposure, rinse abundantly and consult a physician if any symptoms appear/persist. In case of accidental release in the environment of a high volume of digestate, the local environmental authorities should be contacted, as the digestate at a high volume could be very harmful to local wildlife.

Using digestate for microalgal cultivation

Health and safety recommendations discussed in the above sections should be applied when using digestate as a feedstock for microalgal cultivation, especially when using high volumes of digestate to incorporate into large-scale cultivation systems (i.e. volumes greater than $1m^3$). Raw digestate presents a microbiological risk, therefore, treatments should be applied to remove bacterial communities (the different treatments employed are discussed in section 4.). Hence, a "clean" digestate free of potentially harmful pathogens and bacteria should be used for microalgae cultivation.

Considerations should be made regarding the end products for which the microalgal biomass and/or extracted compounds will be used. Indeed, the choice of digestate and more specifically its origin should be known and considered when producing the microalgae and determining the market for the commercialised products in order to comply with regulations.



RECOMMENDATION

Risks linked to the usage of digestate (in any area) should be thoroughly assessed from a Health and Safety aspect before transporting, storing and manipulating digestate. Furthermore, any digestate used should have appropriate certifications in order to comply with regulatory bodies.

3 BEST PRACTICE FOR DIGESTATE CHARACTERISATION AND ANALYSIS

Considerations for digestate characterisation

Digestate origin and seasonality

Because digestate results from waste treatment, its sourcing and origin will be highly variable and its composition will be highly impacted not only by the type of waste used, but also by geographical location (different waste from different areas in NWE). Seasonality is also a parameter to consider when characterising digestate as its composition is likely to change throughout a year. Consequently, the source of the digestate and the time of year it has been produced should be carefully considered when used for microalgal cultivation as the composition might differ and affect microalgal growth and the production of high value compounds. The literature shows that the main liquid digestate used for microalgal cultivation comes from dairy, pig and poultry manure and litter as well as from food waste (Xia and Murphy, 2016). Within the ALG-AD project, digestate is provided by different AD plants located in France, Belgium and the United Kingdom, allowing composition comparison between digestates from different locations in NWE. The digestate provided by Cooperl Arc Atlantique (France) was sourced from pig manure; digestate provided by an Innolab outsourced AD plant (Pittem, Belgium) was of agricultural plant crops and food waste origin; Langage AD (England) digestate originated from food waste (kitchen waste and dairy factory waste). An extensive analysis of these different digestates has been conducted and is presented throughout this section, and published in Fernandes et al (2020).

Considerations for analysis

There are several aspects to consider before carrying out analysis on liquid digestate. Firstly, health and safety measures should be put in place to cover the manipulation of the digestate (see section 2.), and secondly, methods for analysis should be risk assessed according to the chemicals and equipment used. Indeed, digestate characterisation mainly involves the use of chemical methods potentially including hazardous substances. Furthermore, digestate comes in various forms (e.g. thick sludge, centrifuged liquid, etc.) that will affect the methods used for analysis and require prior manipulation of the products before carrying out these analyses. Finally, digestate is known to be extremely rich in some compounds such as phosphorus or nitrogen, and this will also influence the method used for characterisation and the pre-manipulations employed prior to analysis. It is also recommended to carry out characterisation of the digestate as soon as possible after sampling from a digester, as digestate can stay active and its composition can change over time. If analysis cannot be performed in a timely manner, the digestate should be stored in refrigerated conditions to stop, or at least slow down, its biological activity. Biogas production is also likely to modify the composition and needs to be considered from a health and safety point of view. eyes. Users should be working in a well-ventilated location in case of biogas formation, but if working in a confined space, respiratory protection should be used in order to avoid inhalation of harmful gases that could have formed during the digestate transport or storage. When working with a high volume of digestate, for example moving or displacing the digestate, protective footwear (e.g. rubber boots) should be worn to avoid contact with skin or clothes in case of spillage.

Parameters of interest

Nitrogen

Nitrogen (N) content of digestate can be highly variable, and this is mainly due to the diversity of feedstock used in the anaerobic digestion process. Indeed, the source of the digestate has the potential to influence its composition dramatically. Having said that, nitrogen content of the digestate tends to be very high with concentrations going up to 6850 mg/L (Table 2). This factor should be carefully considered when growing microalgae using digestate as a feedstock, because nitrogen is a limiting factor for microalgal growth (Andersen, 2005; Fuentes-Grünewald et al., 2012). However, high concentrations of nitrogen and especially total ammoniacal nitrogen (TAN) can be toxic for microalgae, for example, it has been reported that Scenedesmus sp. could only tolerate up to 500 mg/L of TAN (Park et al., 2010). Consequently, levels of nitrogen should be accurately assessed prior to microalgal cultivation. Several methods are available for total nitrogen analysis, and for some of these methods, digestate should be pre-treated according to analytical requirements to obtain accurate result. Dry matter and particles should be removed by filtration and/or centrifugation and the supernatant should be diluted in accordance with the range of the analytical method used and by considering the high nitrogen content of the digestate. Most studies use colorimetric methods to assess the total nitrogen content, and more precisely they use nutrient kits that allow the manipulation of a minimum amount of chemicals and provide fast and cost-efficient reactions, which is an advantage for routine analysis. However, nutrient kits can lack accuracy and more exact methods of analysis such as the Kjeldhal or Dumas method can be used to obtain more precise measurements of the total nitrogen content. Nevertheless, these methods are costly and require specialist equipment. Hence, these methods of analysis are generally outsourced to specialist laboratories. Analysis can also be realised by segmented flow nutrient analysis or ion chromatography, but these particular techniques require expensive equipment. Within the ALG-AD project, nitrogen content of digestate provided by Langage AD, Cooperl and an Innolab outsourced Ad plant were 6850 mg/L, 2480 mg/Kg and 2430 mg/Kg (liquid fraction of digestate) respectively (details of analysis can be found in Table 2).

Nitrogen and Phosphate composition of digestate provided by Langage AD (UK), Cooperl Arc Atlantique (FR) and an Innolab outsourced AD plant (BE) and associated analytical methods. The details of the filtration and centrifugation treatments of digestate are described in section 4.

Digestate provider	Nitrogen Content	Phosphate content	N to P ratio	Method for analysis
LANGAGE AD (Analysis performed by Swansea University)	Raw: 6850 mg/L Treated (filtration): 4474 mg/L	Raw: 183 mg/Kg Treated (filtration): 135 mg/Kg	Raw: 37.4 Treated (filtration): 33.1	Total Nitrogen: Ammonium Kits-colorimetric methods (Hach 2714100; LCK302; LCK303) and Segmented flow nutrient analyser (AA3-SEAL Analytical). Total Phosphorus: Total Phosphate Kits-colorimetric methods (Hach 2767245) and XRF
COOPERL ARC ATLANTIC	Treated (centrifugation): 2480 mg/Kg	Treated (centrifugation): 138 mg/Kg	Treated (centrifugation): 17.9	Kjeldhal method
INNOLAB outsourced AD plant (Analysis performed by Innolab)	Raw liquid fraction: 2430 mg/Kg Treated (paper filtration): 2370 mg/Kg	Raw liquid fraction: 25 mg/Kg Treated (paper filtration): 9.61 mg/Kg	Raw liquid fraction: 96 Treated (paper filtration): 246.62	Total Nitrogen : Kjeldhal Method; Total Phosphorus : ICP-OES

Phosphate

Phosphate (P), while not being a limiting factor for microalgal growth (Andersen, 2005), remains an important nutrient with the potential to significantly improve microalgal production in cultivation systems. Indeed, extensive studies have been puplished on the bioavailable nitrogen to phosphorus ratio and shown that this ratio was an important parameter to consider when growing microalgae. The optimal ratio of 16 should be reached to produce high biomass volumes (i.e. Redfield ratio, Geider and La Roche, 2002; Rhee and Gotham, 1980). A review of literature showed a highly variable phosphate content in different types of digestate going from 5.1 mg/L (Labbé et al., 2017, digestate source: cattle farm waste) to 716 mg/L (Massa et al., 2017, digestate source: buffalo farming wastewater enriched with whey). As with nitrogen content, there are several methods available for phosphate analysis and colorimetric methods combined with nutrient kits are the most commonly used. Ion chromatography can also be used, but remains an expensive method for analysis. Phosphate content of digestates were 183 mg/Kg, 138 mg/Kg and 25.3 mg/Kg, for the digestate provided by Langage AD, Cooperl and an Innolab outsourced AD plant respectively (Table 2). Low phosphate concentration can be explained by the AD process itself. Indeed, the NWE soil is already very rich in phosphate and AD facilities adjust their processes to reduce the phosphate content in digestate, avoiding a further enrichment of the soil.

RECOMMENDATION

Nitrogen and phosphate contents are important factors to measure in digestate, especially when considering using digestate as a feedstock for microalgal cultivation. Accurate methods should be applied to obtain accurate measurements (i.e. Kjeldhal method). However, in the case of routine measurements, nutrient kits are more suitable because of their lower cost and ready to use type of analysis. If using kits, the range of analysis should be carefully selected in order to reach the most accurate result.

Heavy metals

Heavy metals are commonly present in digestate as they can be found in various concentrations in the different wastes treated by anaerobic digestion (Kupper et al., 2014). Elements such as copper, zinc or iron can be found in trace amounts in digestate and they are valuable compounds (i.e. oligo-elements) that can improve microalgal cultivation when found in the right concentrations (Kropat el al., 2011; Pringsheim, 1949). However, when in high concentration, these compounds can be harmful for the microalgae and can be above the maximum tolerable dietary limits if used in food or feed products (Papadimitriou et al., 2008).

Therefore, it is crucial to measure and monitor the heavy metal content in the digestate before using it as a medium for microalgae cultivation. Detailed analysis of the elemental composition throughout the seasons of the digestate provided by the AD plant Langage AD can be found in Table 3, and are published in Fernandes et al. (2020). These results were obtained by X-Ray fluorescence analysis (Swansea University) and outsourced to a specialist laboratory (NRM laboratories).

Heavy metal composition of raw digestate provided by Langage AD (UK) and an Innolab outsourced AD plant (BE) analysed throughout seasons and associated analytical methods.

Digestate provider	Elemental composition	Method for analysis
LANGAGE AD (Analysis performed by NRM laboratories)	**2016:** Cd: 0.02; Cr: 0.78; Cu: 3.86; Pb<0.5; Hg<0.05; Ni: 0.45; Zn: 5.93	BS EN 15587 - Digestion for the determination of selected elements in water. Aqua regia digestion. (standard by British Standard / European Standard / International Organization for Standardization)
	February 2018: Cd< 0.05; Cr: 2.2; Cu: 6.6; Pb<0.05; Hg<0.05; Ni<0.05; Zn<0.05	
	June 2018: Fe: 488; Mo: 0.38; Cu: 2.75; Mn: 4.76; Se: 0.24; Ni: 1.83; Zn: 8.87; Co: 0.25	
	July 2018: Fe: 527; Mo: 0.36; Cu: 2.58; Mn: 4.58; Se: 0.21; Ni: 1.65; Zn: 9.84; Co: 0.21	
LANGAGE AD (Analysis performed by Swansea University)	**August 2018:** Al: 109.3; Ti: 74.1; Cr: 0.8; Mn: 3.5; Fe: 513.3; Co: 1.7; Ni: 1.4; Cu: 3.1; Zn: 9.5; Se: 0.2; Br: 3.4; Rb: 2.0; Sr: 7.9; Pb: 1.5	X-Ray Fluorescence
INNOLAB outsourced AD plant (Analysis performed by Innolab)	**December 2018:** Zn: 12.6 ; Pb: 2.53; Ni: 0.359; Hg: 0.993; Cu: 2.53; Cr: 1.26; Cd: 0.253; As: 2.53	ICP-OES/ ICP-AES

Dry matter and particle size

In this document, dry matter is described as the amount of solids present in the digestate and this parameter can be highly dependent on the digestate sourcing (i.e. type of waste) and on its processing. Indeed, the anaerobic digestion process can be divided into two separate routes: the wet path and the dry path. The wet path is the most commonly used by AD facilities (85% of utilisation) and it involves the use of a high amount of liquid waste, facilitating the AD process by reducing the amount of organic matter to degrade. The dry path involves the use of a higher amount of solid waste and the resulting digestate usually contains 25 to 45% of dry matter. In this document, only digestates resulting from the wet path have been investigated.

Raw liquid digestate sampled from the digesters and not submitted to any kind of treatment will present a high dry matter content (e.g. dry matter content of 6% in the digestate provided by Langage AD). Dry matter is commonly measured by filtering or drying out the liquid fraction and drying the remaining solid fraction for weighing. It is recommended to measure the dry matter content of digestate aimed to be used for microalgal cultivation as the amount of solids present in the digestate will determine which method is more suited to process the digestate prior to its utilisation in microalgal cultures. Furthermore, some of the compounds of interest for microalgal cultivation, such as phosphate, can be bound to the solid fraction (Gerardo et al., 2015), which will also influence the method used to pre-treat digestate when aiming to release these valuable compounds.

It is also recommended that the particle size distribution in the digestate is assessed. The particle size distribution represents the respective percentages of the different sizes of particles found in the digestate. This can be performed using traditional methods such as a filtration tower with a series of filters with decreasing mesh sizes, or it can be carried out with specialist equipment using laser diffraction. Analysis of digestate particle size distribution is a good indicator to determine filtration systems for digestate treatment. Detailed particle size analysis of the digestate provided by Langage AD can be found in Appendix 3.

Other parameters

Many other parameters can be monitored in digestate, pH for example should be measured routinely as it can be an indicator of changes in the digestate, and can also be a tool for modifying the digestate composition (see section 4.5. for more details). pH in the digestate is also a good indicator of the enzymatic activity in the digesters. Furthermore, as pH plays an important role in microalgal cultures, it should be carefully considered when adding digestate (especially in high volume) to cultures as the digestate pH could potentially affect the culture pH and cause disturbance to the microalgae development. A wide range of probes are available on the market for fast on-site pH measurements.

It is also important to monitor certain macro-elements in digestate, as some of them can be valuable for microalgal cultivation. For example, potassium is found in high concentrations in digestate and is a known fertilising compound (Levine et al., 2011). In the literature, potassium concentrations ranged between 102 and 3389 mg/L in various digestates; the potassium concentrations of digestates described in this document can be found in Table 4. Calcium is another macro-element worth characterising in digestate as it can be a valuable compound in microalgal growth; however if present in too high concentration, calcium can cause precipitations in the cultivation system, decreasing light access for growing microalgae (Harun et al., 2010). Furthermore, calcium contributes to the formation of calcium carbonate, which facilitates biofilm formation in photobioreactors systems. Calcium content of the digestates can be found in Table 4.

Volatile fatty acids (VFAs) should also be considered for digestate characterisation. Indeed, VFAs are mainly produced during the anaerobic process and can be found in high amounts in digestate (Weiland, 2010; Huang et al., 2016). VFAs can be valuable for microalgal cultivation as they are a source of carbon available for microalgae. Consequently, VFAs could be an economically interesting carbon supply for heterotrophic or mixotrophic microalgae cultures (Moon et al., 2013). In the literature, VFA contents of 697.1 mg/L to 13958 mg/L were found (Markou, 2015; Olguin et al., 2015).

Dry matter, pH and macro-element composition of digestate provided by Langage AD (UK), Coopel Arc Atlantic (FR) and an Innolab outsourced AD plant (BE).

Digestate provider	Dry matter	pH	Macro-element composition	Method for analysis
LANGAGE AD (Analysis performed by NRM laboratories)	2016: 5.53%	8.1	2016: K: 1773; Mg: 126; S: 384; Na: 1496; Cl: 3190 mg/L	BS EN 15587 - Digestion for the determination of selected elements in water. Aqua regia digestion. (standard by British Standard / European Standard / International Organization for Standardization). BS EN 14346-Characterization of waste. Calculation of dry matter by determination of dry residue or water content
	February 2018: 2.43%		February 2018: K: 1604; Mg: 29; S: 135 mg/L	
	June 2018: 5.94%		June 2018: K: 1910; Ca: 6302; S: 450 mg/L	
	July 2018: 5.97%		July 2018: K: 1988; Ca: 6310; S: 476 mg/L	
LANGAGE AD (Analysis performed by Swansea University)	3%	7.87	August 2018: Na: 1150; Mg: 113.7; Si: 189.3; S: 227.3; Cl: 918.3; K: 1363.3; Ca: 1683.3 mg/Kg	X-Ray Fluorescence. Filtration and dry matter weighing.
COOPERL ARC ATLANTIC	0.78%	8.0 - 8.1	Ca: 188; Mg: 26.4; Na: 426; K: 1054 mg/kg	NA
INNOLAB outsourced AD plant (Analysis performed by Innolab)	1.23%	7.87	Ca: 146; Mg: 133; K: 2130 mg/kg	ICP-OES/ICP-AES

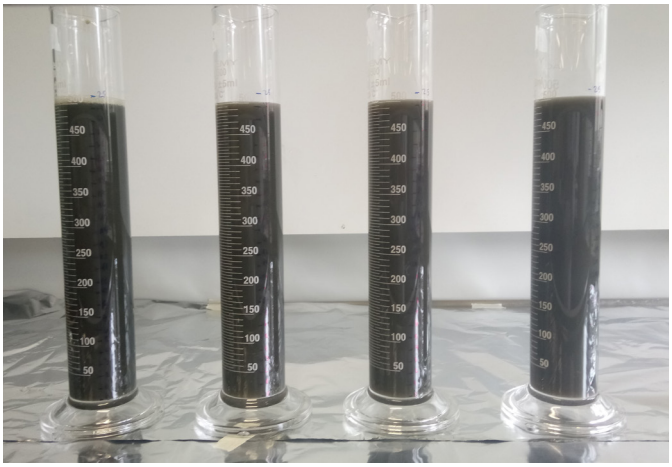
RECOMMENDATION

Ideally, a full characterisation of digestate should be performed before using it as feedstock in microalgal cultivation system. Many methods are available for analysis with a wide range of cost and feasibility and samples can also be sent to specialist laboratories for a full analysis. Nitrogen and phosphate content should be assessed as accurately as possible as they will be determining factors for the quality of growth of microalgae cultivated on digestate. An emphasis should also be made on physical parameters and particularly dry matter and particle size, as these will contribute to the selection of the best digestate treatment before its incorporation into microalgal cultures.

4 BEST PRACTICE FOR DIGESTATE PRETREATMENTS

Liquid digestate appears as a thick and dark liquid (Figure 6) that needs to be processed before being used for microalgae cultivation. Indeed, because of its high content of dry matter and its dark colour, untreated digestate is not suitable for the cultivation of microalgae as it would block the access to transmissible light to the microalgae in culture (Marcilhac et al., 2014). Furthermore, liquid digestate is an ever-changing product, which stays active throughout its life span, and digestate presents a potential for development of existing or new bacterial populations; consequently, a sterilisation treatment should also be applied to digestate prior to utilisation for microalgae cultivation to avoid bringing harmful bacteria to the microalgae cultures.

Methods for treating raw digestate can be very diverse and include processes such as centrifugation, filtration and ultra-filtration, dilution, autoclaving, acidification, alkalisation, settlement or sedimentation, precipitation or flocculation, sterilisation by ultraviolet, sonication or a combination of several of these treatments. The ALG-AD project aims to use digestate as a feedstock for high scale cultivation of microalgae, and hence remove the cost of nutrient supplementation to the cultures. Consequently, the pre-treatment methods of digestate presenting the best cost-efficient options were investigated within the framework of this document.



Settlement

Raw digestate was provided by the anaerobic digestion plant Langage AD (Devon, UK) and sampled straight from the digester. The liquid digestate could be qualified as a dark coloured thick sludge (Figure 6). The digestate was left to settle overnight to allow separation of the solid and liquid fractions. However, after four hours of settlement, both fractions were still homogenous. This first settlement trial was realised on a large volume of digestate (i.e. 500L), which could explain the slow settlement rate. However, when settlement was tested on a smaller volume (i.e. 40 mL), similar results were observed and the digestate did not appear to settle, even for a settlement time of approximatively 4 days.

Settlement alone did not provide conclusive results for the digestate provided by Langage AD, and the homogeneity properties of this digestate are probably the main reason for the slow settlement speed observed. However, settlement should not be disregarded as a pre-treatment of digestate prior to microalgae cultivation as it could provide a cost-effective solution if tested on different types of digestate. Indeed, settlement has the potential to enhance and facilitate other types of pre-treatments, such as dilution and filtration. Furthermore, settlement has been reported in the literature to be an efficient and cost-effective treatment to separate liquid and solid fractions in digestate. For example, Godos et al. (2009) showed that a digestate sedimentation at a residence time of five days reduced the total of suspended solids by 70%. In addition, when using sedimentation as a pre-treatment of digestate, an adequate pumping system should be put in place in order to collect the unsettled layer of liquid and avoid mixing of the separated fractions.

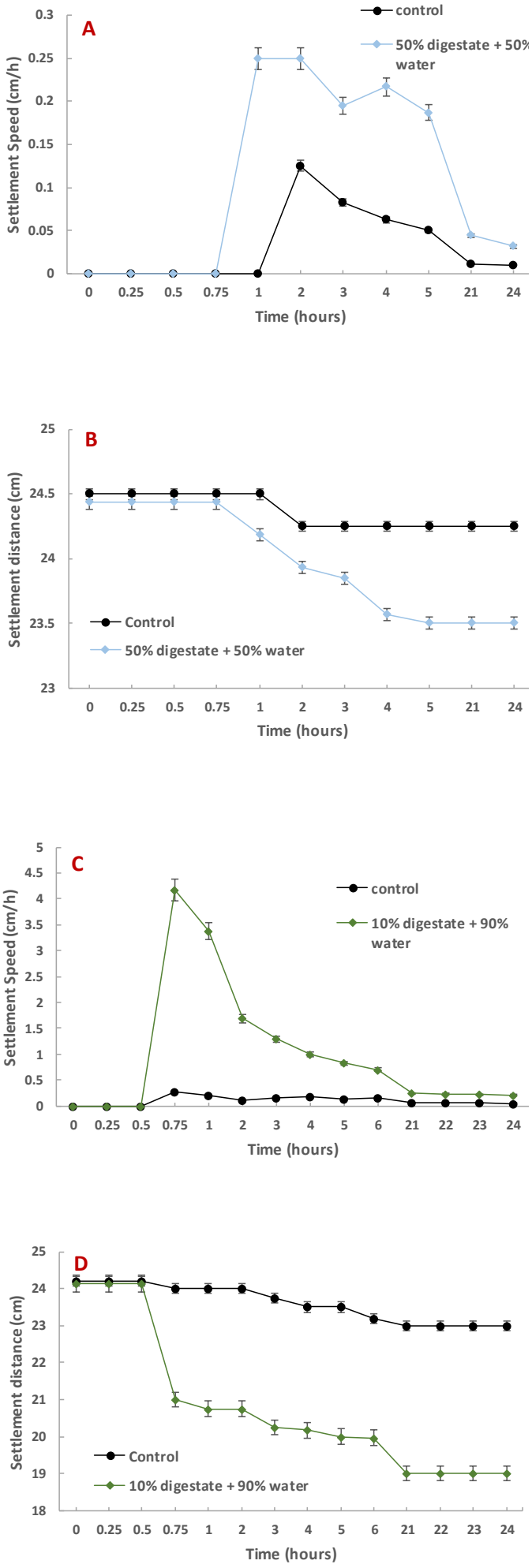
Dilution and Settlement

Several studies have used a combination of settlement and dilution in order to pre-treat liquid digestate and reduce the amount of solids to facilitate microalgal cultivation. Dilution of digestate helps solubilising mineral precipitates in the digestate and potentially releases compounds of interests for microalgae cultivation (Wahal and Viamajala, 2016). Indeed, previous results presented in this document showed that most of the phosphate present in digestate was bound to the solid fraction (Fernandes et al, 2020). Consequently, dilution would be an efficient and cost-effective treatment to release this precipitated phosphate and make it available for microalgal growth. This release would provoke a modification of the N to P ratio, which is an essential parameter for microalgal growth.

In this section, the settlement speed as well as the composition of digestate were investigated for several dilutions of digestate with deionised water. A 10% digestate and 50% digestate mixed with water dilutions were tested in measuring cylinders. The settlement speed of the digestate-water mixture was assessed over a 24-hour period and the settlement layers were sampled for nitrogen, phosphate, heavy metals and particle size analysis (details of the experimental design can be found in Appendix 4).

Settlement Rate

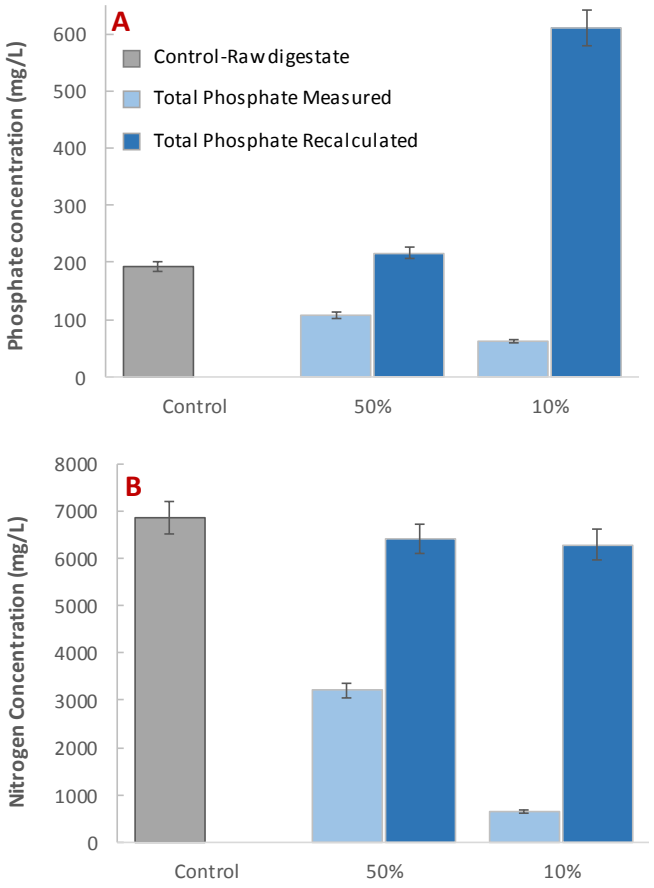
This trial showed that settlement occurred at a faster rate for a 10% digestate/90% water mixture compared to a 50% diluted digestate (Figure 3). Over 24 hours, the settlement speed was of 0.107 cm/hour for a 50% digestate mix, and of 0.993 cm/hour for a 10% digestate mix. This result was expected, as a higher dilution rate decreases the amount of particles present in the settling mixture and the added water potentially helps dissolving precipitates present in the digestate. Furthermore, observations showed that the 50% mixture settled mainly between 45 minutes and 4 hours while the 10% mixture settled mainly between 45 minutes and 2 hours (with a maximum settling speed of 4.18 cm/hours measured at 45 minutes after the trial started) (Figure 3).



Settlement speed (A) and settlement distance (B) measured for a 50% diluted digestate mix (Blue lines) and 100% digestate control (black lines). Settlement speed(C) and settlement distance (D) measured for a 10% diluted digestate mix (Green lines) and 100% digestate control (black lines).

Nutrient Content

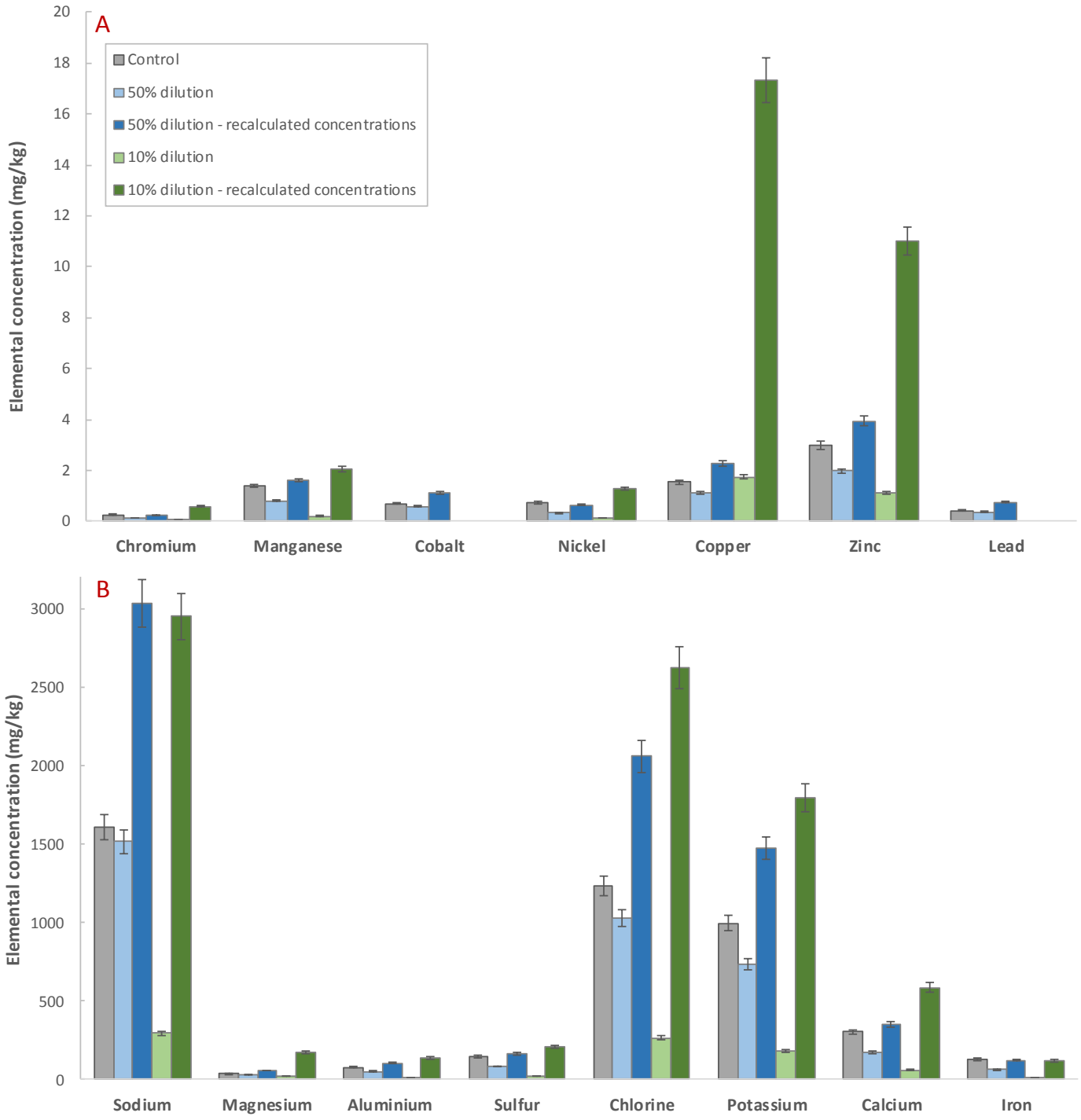
Analysis of nitrogen, phosphorus and heavy metals also took place in this dilution/settlement trial. Nitrogen was relatively high in the control (i.e. 100 % concentration of digestate) with a concentration of 6850 mg/L, which is expected for a nutrient rich digestate and this result was comparable with results from the literature (Kumar et al., 2010; Markou, 2015; Wang et al., 2010; Zhou et al., 2012). Values of total nitrogen for the 10% and 50% digestate mixtures showed consistent results and were comparable with the nitrogen content of the pure digestate (Figure 4 (B)). These results demonstrate that diluting digestate was not significantly affecting the total nitrogen content of the digestate. On the other hand, the recalculated phosphate content of the 10% digestate mixture was three-fold higher than the phosphate concentration of the pure digestate (192.5 mg/L and 609.6 mg/L in the raw digestate and 10% digestate respectively) (Figure 4 (A)). This result showed that dilution allowed the dissolution of some of the phosphate bound to the solid fraction, which was consistent with previous results (Wahal and Viamajala, 2016). This nutrient analysis is very valuable with regards to microalgal cultivation as it revealed that phosphate content can be modified in the digestate by using a process as simple as water dilution. Indeed, a modification of the phosphate content allows a shift of the N to P ratio, which is an essential parameter to consider when cultivating microalgae. In this specific trial, dilution allowed to change the N to P ratio from 35.5 to 10.3, which is closer to the optimal Redfield ratio of 16 for microalgal growth (Tett et al., 1985; Geider and La Roche, 2002).



Phosphate (A) and nitrogen (B) content in 100% digestate control (in grey), 50% and 10% digestate (light blue) and recalculated values for 50% and 10% digestate (dark blue)

Heavy metals content

Heavy metals are also an important parameter to monitor as they can be toxic in high concentrations, but can be essential oligo-elements for microalgal growth when found in trace amounts (Kropat el al., 2011; Papadimitriou et al., 2008; Pringsheim, 1949). The elemental analysis of the two and tenfold diluted digestate (XRF analysis) revealed that elements such as nickel, copper, lead, zinc or Iron were found in trace amounts in the raw and diluted digestate (Figure 5 (A)). On the other hand, elements such as sodium, potassium or calcium were found in higher amounts for all digestate concentrations (Figure 5 (B)).



Heavy metal composition (A and B) in 100% control (grey), 50% diluted digestate (light blue) and recalculated values (dark blue) and for a 10% diluted digestate (light green) and recalculated values (dark green)

Furthermore, our results showed that most elements were found in higher concentrations in a 10% dilution when values were recalculated. This showed that, as for the phosphate content, dilution allowed the dissolution and release of most elements, making them available for microalgal cultivation. Heavy metals with a potential toxicity were also released by dilution (copper, nickel, etc.). However, most elements were still measured in trace amounts, and this should be monitored in order to avoid toxicity in the microalgal cultures.

Particle size Analysis

Particle size analysis were conducted using a Malvern master sizer particle analyser. Analysis was conducted on samples after 24 hours of settlement. Results showed a similar trend for a 100% digestate control and for both 10% and 50% dilutions. Indeed, analysis showed that a higher percentage of big particles (50 to 100+ µm) were mainly found in the lower layers of the settled digestates and that smaller particles (0.1 to 50 µm) were evenly distributed in all the layers of the settled digestate (See Appendix 5 for detailed results). This result showed that bigger particles tended to sink faster in a settling digestate and are found mainly in the bottom layers of digestate. Consequently, if the upper layers of a settled digestate are collected, most of the large particles will be left in the bottom layers, allowing this settlement treatment to be combined with adequate filtration systems more easily.

This trial showed that settlement and dilution were two digestate treatments that when combined, could 1) increase the settlement speed of the digestate allowing a faster separation of the liquid and solid fractions and hence a faster processing of the digestate for microalgal cultivation purposes; 2) dilution has a high potential for release of valuable elements in the liquid fractions, such as oligo-elements and nutrients, making them available for microalgal uptake.

Most importantly, this trial showed that the nutrient content of the digestate and especially the phosphate content could be altered, provoking a modification of the nitrogen to phosphorus ratio allowing it to be closer from the optimal value of 16 aimed in microalgal cultivation systems.

RECOMMENDATION

Information pulled from this trial showed that a 10% dilution is the best in terms of settlement speed when processing digestate and in terms of obtaining the best content regarding nutrients and oligo-elements for microalgae cultivation. Hence, dilution should be considered when treating digestate and the level of dilution should be regarded as a function of nutrient and heavy metals release in order to suit specific microalgal species. However, when using dilution and/or settlement, the nature and sourcing of the digestate should be thoroughly considered, as the physical properties of a digestate are likely to change with these parameters.

Centrifugation

Centrifugation can be an efficient process in order to separate the liquid fraction (i.e. supernatant) and the solid fraction in a liquid digestate and provide a clear feedstock for microalgal cultivation. There are many parameters to consider when centrifuging a liquid, such as the rotation speed (or relative centrifugal force (rcf)), the temperature and the centrifuging time. Studies have used centrifuging to treat their feedstock for microalgal cultivation (Singh et al., 2011; Cheng et al., 2015; Markou, 2015; Marcilhac et al., 2015; Tao et al., 2017) and details of the centrifuging parameters used can be found in Appendix 8. In this section, the centrifugation processes used by the two AD plants Cooperl Arc Atlantique (France) and an Innolab outsourced AD plant (Belgium) will be described.

The digestate produced by Cooperl Arc Atlantique is sourced from pig manure and is continuously centrifuged. The centrifugation is realised by mechanical decantation, allowing a separation of the solid and liquid fractions. The process is facilitated by added polymers inducing the flocculation of the solid fraction particles (the detailed process is described in Appendix 2 (2)).

The digestate provided by the Belgian AD plant is sourced by a mixture of food and plant waste. Once produced, the digestate containing 9.8% of dry matter is centrifuged to separate liquid and solid fractions. A flocculation process is then implemented by adding polymers and iron sulphate (i.e. coagulant)in order to precipitate the particulate phosphorus. The remaining liquid fraction of digestate goes through a dissolved air flotation process (DAF) allowing the removal of suspended matter such as oil or solids. A reverse osmosis step is then implemented by addition of acid (H2SO4). The resulting digestate is very rich in nitrogen and potassium with a dry matter content of 1.23%.

As described in this section, the centrifugation process is often associated with further processing steps, such as flocculation or decantation in order to facilitate the separation of liquid and solid fractions in digestate. Combining several methods for the treatment of digestate has been reported in many studies (Zhou et al., 2012; Cicci and Bravi, 2014; Xu et al., 2015; Ledda et al., 2016b) as this provides the best results in terms of solids removal and in some cases, can provide higher contents of nutrients and other compounds valuable for microalgal cultivation (Wahal and Viamajala, 2016).

RECOMMENDATION

While centrifugation is an efficient method for treating digestate and separating liquid and solid fractions to facilitate its use as a feedstock for microalgae cultivation, this process can be costly when used for high volumes of digestate and requires a high amount of energy that can affect the viability of the digestate upstream process in microalgal cultivation systems. Consequently, centrifugation should be carefully considered if used to process digestate continuously in order to feed microalgal cultures in large-scale cultivation systems. The centrifugation process seems better suited for small-scale cultivation systems rather than pilot or commercial scale.

Paper filtration bag

Filtration

Paper Filtration

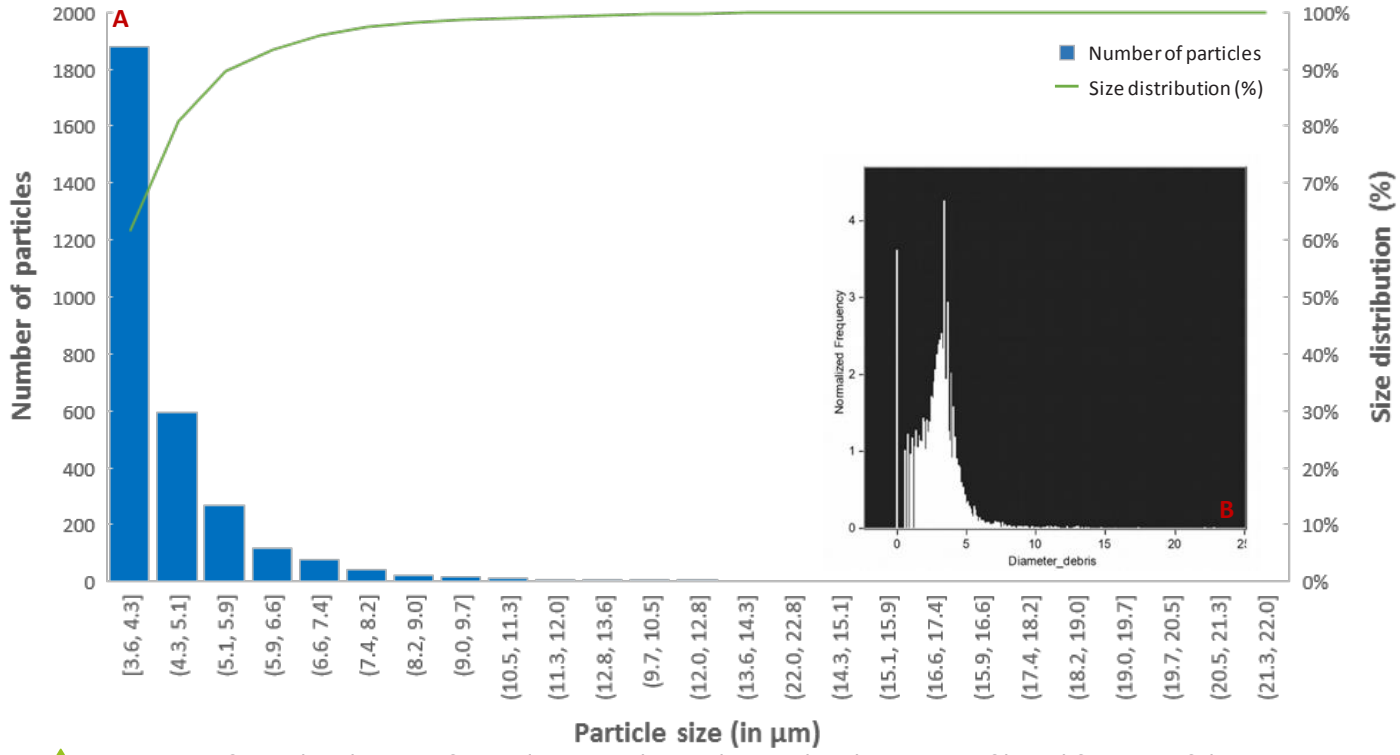
Paper filtration has the potential to be an efficient technique to filter the liquid fraction (also called thin fraction) of digestate due to this fraction low viscosity (dry matter < 2%). This process uses cellulosic filter paper and is operated using a rolling drum filter or a belt press filter in both pressurized and unpressurized conditions. Once used, the filter paper can be digested in the AD plant, improving the sustainability of the pre-treatment process. The thin fraction of digestate obtained from Innolab outsourced AD reactor was collected following on-site treatment to precipitate particulate phosphorus, reducing its dry matter content from 9.8% to 1.23%, as described in section 4.3. This liquid fraction was further processed by paper filtration in order to remove larger suspended particles (i.e. superior to 10 µm) that could have a negative effect on light accessibility for microalgae, an important parameter for photosynthesis. When using a filter paper with a pore size of 4-11 µm, the dry matter content was reduced from 1.23% to 1.18%.

To determine if the used filter paper (pore size: 4-11 µm) was sufficient for removing particles larger than the microalgal cells (5-10 µm), a flow cytometer was used. The particle size distribution can be seen in Figure 6.

The particle size analysis indicates that paper filtration was an efficient pre-treatment to remove most of the particles larger than 5 µm and almost the totality of particles larger than 10 µm from the liquid digestate. Moreover, no significant differences in the pH, electrical conductivity and total nitrogen composition of the liquid fraction were observed following the paper filtration. However, 62% of the total phosphorus content was lost due to the removal of particles binding this element.

The significant loss of total phosphorous during the filtration should not be seen as a disadvantage of the described method due to several factors: (i) the phosphorous content of the liquid fraction before filtration was already low, yielding a N/P ratio of 96; therefore, a phosphorous supplementation would be necessary for microalgal cultivation even without the loss due to filtration; (ii) the lost phosphorous was particle-bound and, therefore, was not in its soluble form and was unlikely to be bioavailable for the microalgae; and (iii) the increase of light penetration and microalgal growth due to the removal of the particles bigger than

the microalgal cells should compensate for the need of a slightly higher phosphorous supplementation. Therefore, paper filtration could be seen as a promising technology for pre-treating the liquid fraction of digestate for microalgal cultivation - however, due to the pore size, there is potential for pathogens to be found in the paper filtered digestate..



Histogram of size distribution of particles (A) and particle size distribution (B) of liquid fraction of digestate post-paper filtration.

Micro-Filtration

Filtration has been widely used for pre-treating digestate used for microalgal cultivation (Ledda et al., 2016a; Wen et al., 2017; Massa et al., 2017; Wang et al., 2010; Park et al., 2010), and this method allows for the removal of most of the particles and large microorganisms (e.g. protozoa) potentially present in the digestate. In recent studies, micro-filtration has also been used as a digestate pre-treatment (Abou-Shanab et al., 2013; Khan et al., 2018; Olguin et al., 2015; Ledda et al., 2016b; Silkina et al., 2017) . Micro-filtration is the filtration of a product through a fine material (called membrane), using a combination of high pressure and small pore size in order to retain any particles but also bacteria, providing a mechanically sterilised permeate (i.e. liquid obtained post ultra-filtration). Membrane micro-filtration has also been used for nutrient recovery from digestate (Gerardo et al., 2015). Here, a ceramic membrane with a pore size of 0.1 µm was used to realise the micro-filtration of raw digestate (Specification of the membrane used can be found in Appendix 6). The obtained permeate appeared as an amber coloured clear liquid (Figure 7).

Micro -filtration can be an efficient and straightforward method to treat digestate prior to microalgal cultivation as it can eliminate both bacteria and residual matter. The process can however be improved, as the permeate flow rate can be significantly slowed down by the thickness and amount of organic and inorganic matter present in the digestate. When treating high volume of digestate with the objective to feed large-scale microalgae cultivation systems routinely, this slow flow rate could become an issue and present an obstacle to commercial production of microalgae using digestate as feedstock. Furthermore, feeding raw digestate in the micro-filtration system involves an extensive cleaning procedure after utilisation, which is a procedure that can be costly and time consuming (the detailed cleaning procedure of ceramic membrane is described in Appendix 6).

Pure digestate pre ultra-filtration (left) and post micro-filtration (right)

The filtration of a pre-diluted digestate has the potential to improve the micro-filtration process. Indeed, as discussed earlier, water dilution of the digestate helps dissolve some of the solids and releases some valuable compounds for microalgal growth. This process would allow the dissolved compounds to remain in the liquid fraction post micro-filtration and it would facilitate the elimination of bacteria and residual particles left in the diluted digestate compared to the direct micro-filtration of a raw digestate. It has been established that a mixture of 10% digestate and 90% water demonstrated good results in terms of settlement speed, nutrient and oligo-element release. Besides,d this dilution provides the best N to P ratio for microalgal growth. Consequently, it would be interesting to investigate if the 10% digestate mixture presents a satisfying flow-rate when ultra-filtered in order to assess the feasibility of the complete upstream process for large-scale cultivation of microalgae.

Different dilution of digestate will be tested on the micro-filtration system installed at the Langage AD facilities (characteristics of this other membrane system can be found in Appendix 6), confirming or informing which dilution is best for pre-treating the digestate. After micro-filtration the resulting digestate is ready to use directly in microalgal cultivation as it has been mechanically sterilised.

Other pre-treatment methods

Many other pre-treatments of digestate can be used, such as autoclaving, acidification, alkalisation, precipitation or flocculation. Autoclaving has been used in some studies in order to sterilise the digestate (Kumar et al., 2010; Hollinshead et al., 2014; Koreiviene et al., 2014; Dickinson et al., 2015), and while this technique is very efficient for small volumes of digestate, it is unrealistic to apply it for industrial scale. Another process used to treat digestate is flocculation, which combined with filtration can facilitate the elimination of the liquid digestate dry matter content (Ledda et al., 2016b; Salati et al., 2017). However, the flocculation process involves the use of chemicals such as iron sulphate, which if used in high volume could be incompatible with large-scale cultivation of microalgae. Furthermore, the flocculation process could provoke the retention of valuable compounds used for microalgal growth.

Alkalinisation and acidification of digestate are also methods used to treat digestate and more specifically to induce the release of nutrients in the liquid fraction as well as to increase the flow rate when filtering digestate using membranes. For example, Gerardo et al. (2015) showed that a pH of 11 significantly increased the permeate flow rate during ultra-filtration and that a basic pH facilitated the extraction of nitrogen and phosphorus during the filtration process. Similar methods have been used in order to prepare digestate used as feedstock in microalgae culture (Ledda et al., 2016b; Silkina et al., 2017) and allowed an increase in nutrients in the treated digestate. However, with the aim to grow microalgae at a large-scale this process seems unrealistic with regards to maintaining a cost efficient production of microalgae at a pilot or commercial scale.

Remaining products

The digestate treatments presented and discussed in the sections above involve the separation of liquid and solid fractions of digestate and the utilisation of the liquid fraction for microalgal cultivation as a feeding medium. However, the solid fraction remains unused and is still rich in nutrients and other compounds described earlier. Here, we suggest that the remaining unused solid fraction could be dried and used as a fertiliser with a lesser concentration of nutrients (as some of the nutrients are contained in the liquid fraction) and be spread on agricultural land. Alternatively, the remaining digestate would be stored in digestate storage and disposed of through the appropriate waste disposal routes.

RECOMMENDATION

Several methods for treating digestate have been described in this section. From an economical point of view while working at pilot or commercial scale, dilution and filtration seem to be the best and most cost-efficient processes. These fall in line with the ALG-AD project objectives of creating a circular economy with limited costs and low generation of waste. However, many treatments presented in this section proved to be efficient at treating digestate and should not be disregarded for small-scale microalgal culture or when their use is cost-permitted. Furthermore, depending on the digestate treated, a combination of several treatments (e.g. settlement/dilution/filtration, flocculation/filtration, filtration/autoclaving, etc.) appear promising for preparation of NRD effluent for use as a microalgal culture feedstock.

For the ALG-AD project, membrane filtration was the most successful in terms of solids and pathogen removal, so if budget permits, this would be the advised method to clean raw digestate

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APPENDICES

Appendix 1. The ALG-AD project

What is ALG-AD?

ALG-AD is an Interreg NWE funded project in which new technology is being developed to take excess waste nutrients produced from anaerobic digestion of food and farm waste to cultivate algal biomass for animal feed and other products of value.

ALG-AD brings together a group of scientists and engineers from 11 different partners in four countries across North West Europe. These academics are working together with industry to develop a circular economy solution to create wealth from waste.

Langage photobioreactor for algal cultivation

Langage AD facilities

Why is the project necessary?

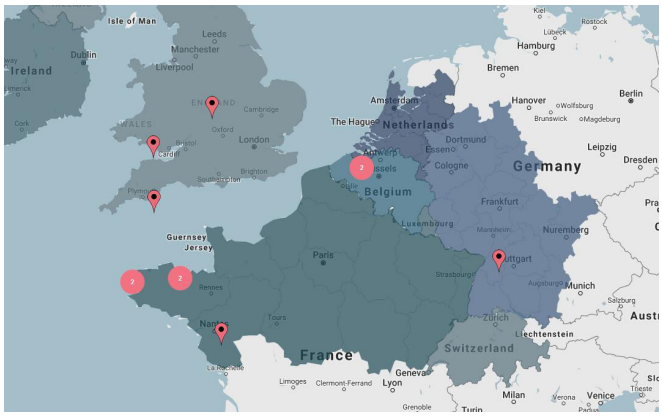
There is an urgent need to develop sustainable food and farming.

North West Europe is a densely populated and intensely agricultural area. It thus contributes disproportionately to food and farm waste produced in the EU each year.

Increasing amounts of food and farm waste are processed using anaerobic digestion (AD). AD converts waste to biogas used for energy and a liquid nutrient rich digestate, most of which is returned to land as a biofertiliser.

However, there are strict limits on the amount of digestate which is allowed to be put back on agricultural land. Strict limits are imposed with EU legislation and so-called Nitrate Vulnerable Zones. This is increasingly creating excess unwanted nutrients.

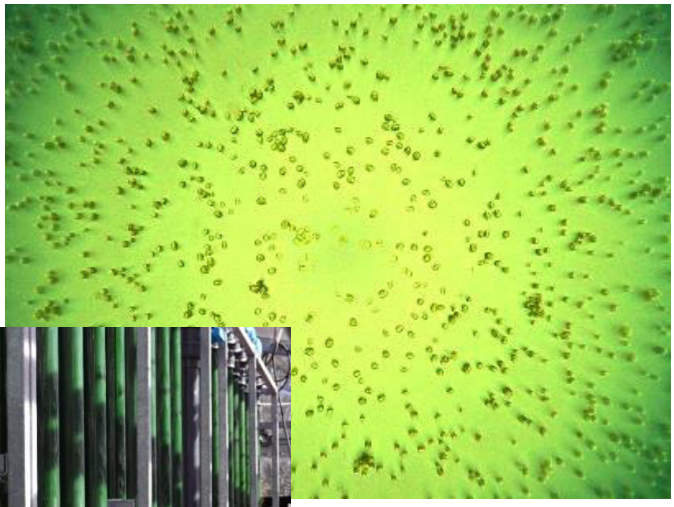
The ability to use these excess nutrients to produce new products presents a circular economy solution.



ALG-AD project partner map

How can ALG-AD help?

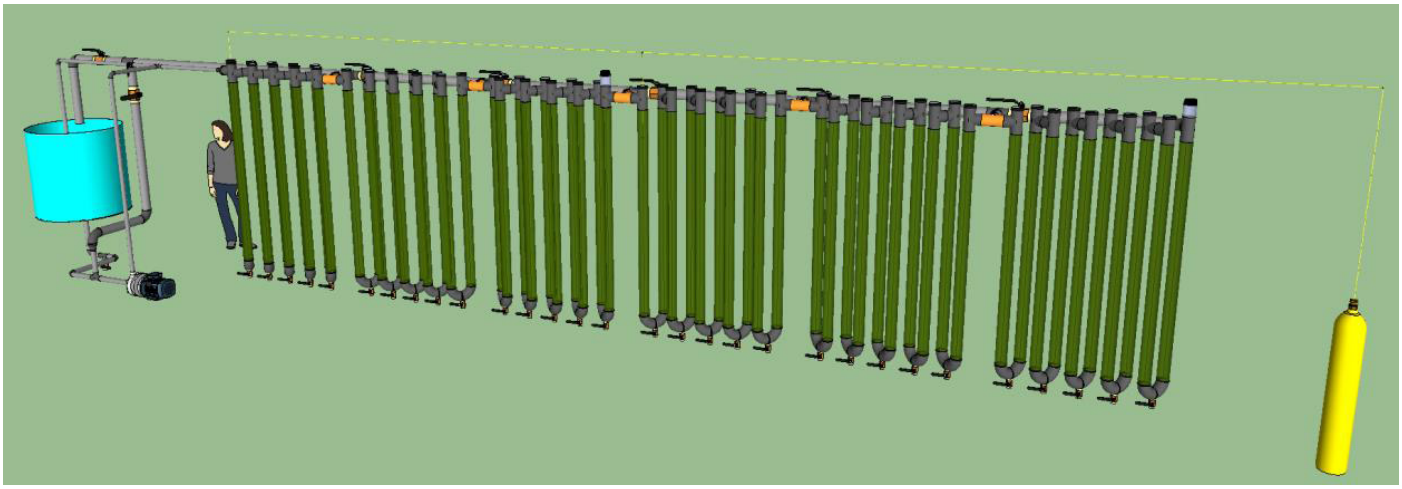
ALG-AD combines algal and AD technology. Microalgae, will be cultivated, converting the unwanted nutrients into biomass. The cultivated algal biomass is rich in protein and other useful compounds, and can be used to generate sustainable animal feed products and other useful bio-products.



Algae in Cultivation

What will ALG-AD be doing?

ALG-AD is building three pilot facilities at 3 distinct 'real life conditions locations in North West Europe: Devon, Ghent and Brittany. Each facility will use local conditions to grow microalgae and record results. Information from the three pilots will be used to generate Decision Support Tools. These tools together with demonstration to stakeholders will promote adoption of the new technology.



Schematic representation of planned installation of algal cultivation system for Langage AD

For more information on the ALG-AD project, contact Carole Llewellyn (project PI, C.A.Llewellyn@Swansea.ac.uk) or Louise Hall (Project manager, l.t.hall@swansea.ac.uk).

www.nweurope.eu/projects/ALG-AD

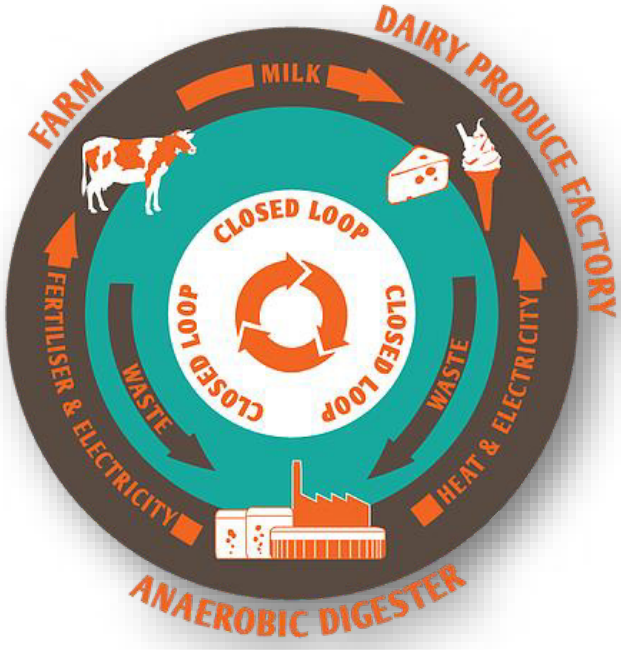
Appendix 2. AD plant facilities

LANGAGE AD

Background

Langage AD is an Anaerobic Digestion plant built by the dairy farm Langage in Devon (http://www.langagefarm.com/). The farm also comprises a dairy product factory. Both produce waste fed into the digesters.

As an innovative way to secure fertility of the farm soils and provide a reliable source of electrical and thermal energy to the dairy products factory, Langage Farm invested in anaerobic digestion (AD) as a means to create a truly closed-loop system of resources between its three businesses.



Closed loop connecting the different branches of the Langage facilities in Devon

Anaerobic digestion process

Anaerobic Digestion uses bacteria to break down organic matter into a methane rich gas. Production waste from the dairy products factory, and food waste from local businesses such as schools and restaurants is utilised as feedstock for the AD plant. The AD plant produces electricity and heat for use in the dairy products factory, and residual biofertiliser is applied to the farmland to grow forage for the dairy farm. The milk returns to the products factory for processing and the cycle begins again. Any surplus electricity generated is exported to the National Grid - Langage AD can power up to 500 households.

Langage AD digestate

Langage AD PAS110 Biofertiliser is a complete liquid fertiliser containing a cost-effective source of N-P-K-S for the agricultural market, which also offers a host of additional benefits not found in a traditional manufactured fertiliser.

Biofertiliser contains many trace-elements important for soil health and crop quality, such as Magnesium, Zinc, Manganese and Boron. Biofertiliser also has a high pH and contains bicarbonate - this significantly reduces the acidification caused by nitrogen application on the soil.

Langage AD facilities

For more information on LANGAGE AD, contact Gary Jones (Technical Director, gary@langagefarm.com).

Cooperl – digestate valorisation facilities

Background

Cooperl Arc Atlantique is a French agricultural group specialised in pig farming and it includes 2700 farmers throughout the French territory.

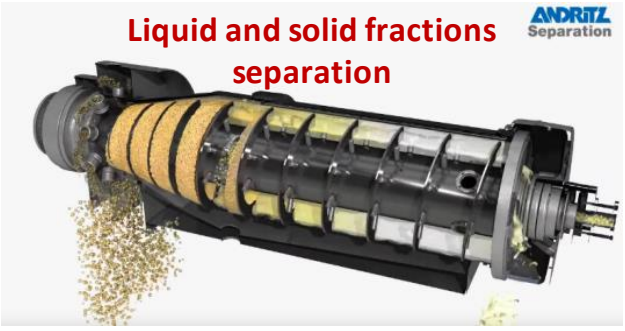
In the 90s, Cooperl Arc Atlantique diversified its activity and created the branch Dénitral which sells, builds and exploits pig waste treatment plants. These plants help provide a solution to farmers to treat the waste produced by their farming activity which is rich in nitrogen and phosphorus.

The treated waste is valorised and used to produce thermal energy and the resulting solid fraction is dried and used as a primary ingredient for the production of natural fertilisers (produced by another branch of Cooperl : Fertival). The fertilisers produced by Fertival are diverse and used for a wide range of crops. All fertilisers formulated are certified Iso 9001 2015- European certification FR 22 261 004.

Effluent treatment

After the anaerobic digestion process, the resulting digestate goes through a series of chemical and mechanical treatments in order to separate solid and liquid fractions. A centrifugation step is realised continuously with the addition of polymers to induce flocculation of the solid matter and facilitate the centrifugation process. The centrifugation consists in a mechanical decantation aiming to separate solid and liquid fraction.

https://www.youtube.com/watch?v=OqEODWcJwnY.



Mechanical decantation process for digestate centrifugation

For more information on Cooperl Arc Atlantique, contact Barbara Clement-Larosiere (R&D project manager, barbara.clement-larosiere@cooperl.com)

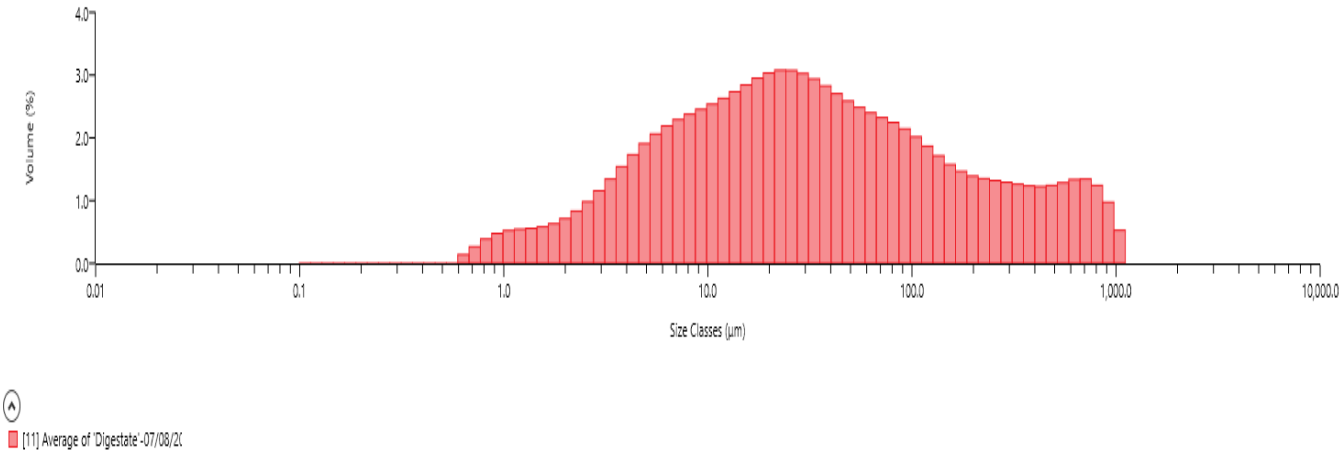
Appendix 3. Particle size analysis of Langage AD digestate

Particle size analysis have been realised with a Malvern Master sizer instrument. This equipment uses laser diffraction to measure the size of particles. It measures the intensity of light scattered as a laser bean passes through a dispersed particulate sample. Data are then analysed to calculate the size of the particles, creating a scattering pattern.

In total, three samples of raw digestate were analysed through the instrument, using a sample volume of 4 mL diluted in 500 mL of water. The results showed a Normal distribution of the particle size in the raw digestate with 28.27% of particles with a size of 0.1 to 10 µm; 34.45 % of particles with a size of 10 to 50 µm; 13.62 % of particles with a size of 50 to 100 µm and 23.65% of particles with a size higher than 100 µm.

Particle Analyser (Malvern master sizer)

Histogram of the particle size distribution in raw digestate

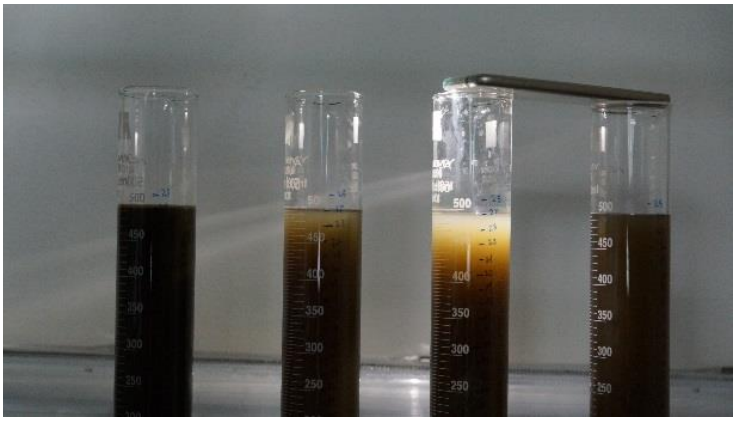


Appendix 4. Detailed experimental design of Settlement Experiment realised at Swansea University

Raw digestate diluted at 50% (50% water + 50% digestate) and at 10% (90% water + 10% digestate) mixtures were prepared and left to settle in measuring cylinders (volume: 500 mL) in a fumehood in order to limit exposure to potential biogas formation during the experiment.

Experimental set-up

Each dilution was tested in triplicate and a raw digestate control was studied. All the digestate/ water mixtures were left to settle for 24 hours with regular metric measurements. These measurements were realised by shedding a light on top of the measuring cylinders in order to observe the layers of settling digestate.

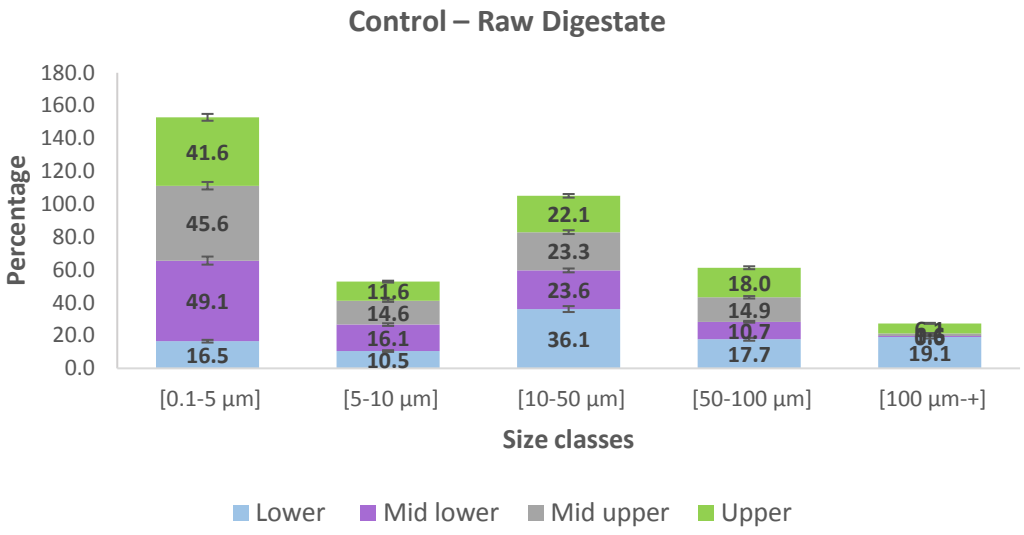


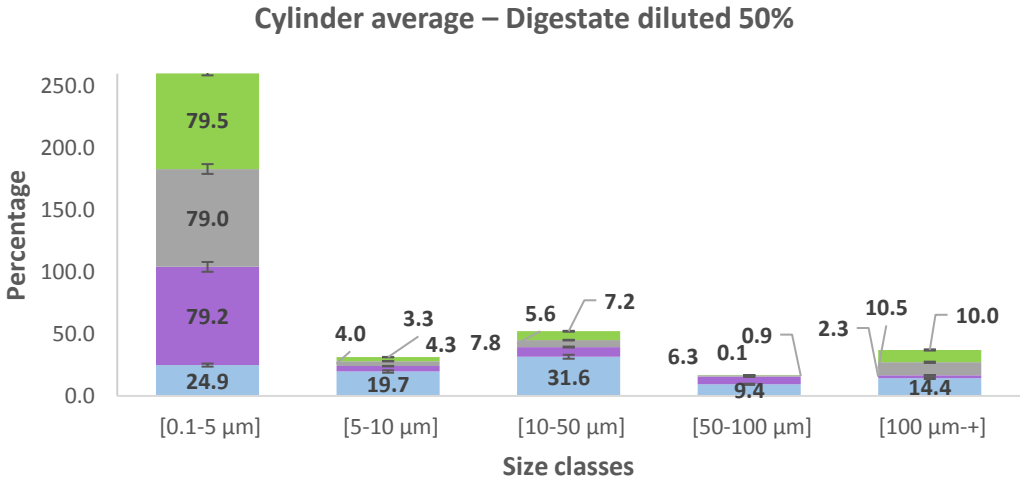
Settling layers of digestate-metric measurements

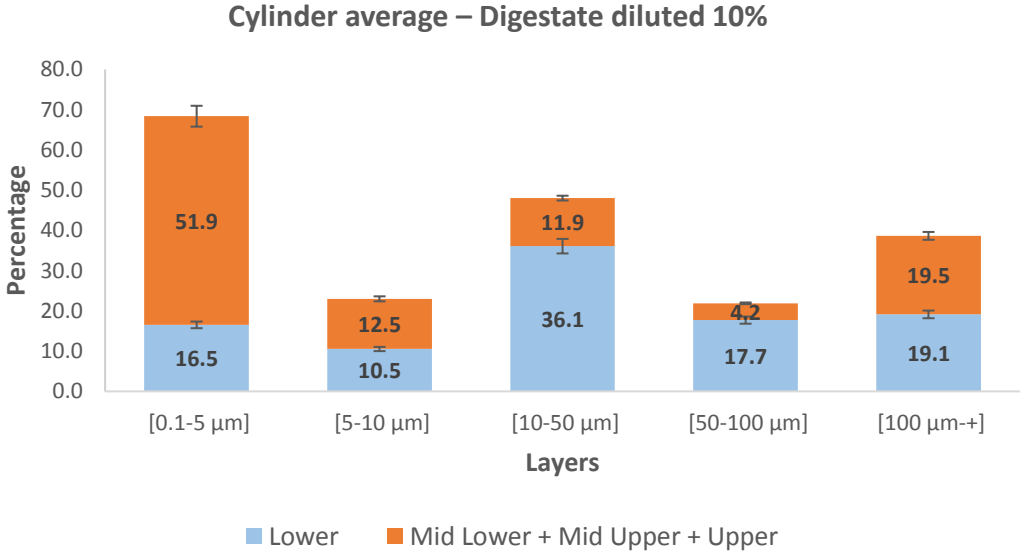
After 24 hours of experiments, samples were taken by pipetting the different settled layer as follow: bottom layer, mid-bottom layer, mid-upper layer and upper layer. Samples were stored at 4°C before analysis.

Nitrogen and phosphate measurements were realised with nutrient kits (HACH-LANGE) using colorimetric methods. Samples were centrifuged 13 minutes at 10 000 rpm in order to separate liquid and solid fractions of the digestate and analysis were conducted on the supernatant after appropriate dilution in order to match the nutrient kits ranges. Phosphate and heavy metal content were also analysed using an X-Ray fluorescence instrument. Particle size analysis was realised using a Malvern Master sizer (see description in Appendix 3).

Appendix 5. Detailed results of settlement experiment (particle size)







Appendix 6. Membranes Specifications and cleaning procedure

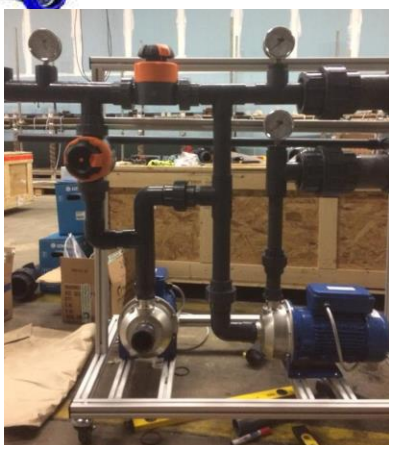
Ultra-filtration membrane description

Swansea University trials realised on digestate ultrafiltration were conducted on a 0.1 µm pore size ceramic membrane using a pressure ranging from 1.5 to 2.5 bars.

The entire set-up comprised a 60L capacity tank connected to a pump sucking in the digestate into the membrane for filtration. The permeate is collected at one end of the membrane and the remaining sludge (or retentate) is pumped back into the tank where it is mixed with the remaining digestate to be filtered. Ultra-filtration is realised up to the point where the volume left in the tank has reached roughly 10- 15 L. The remaining retentate is a very thick and concentrated sludge.

The ultra-filtration process installed in the Langage AD facilities will be set-up in line with the photobioreactor system for large-scale algal cultivation. The ultra-filtration of digestate will be realised via a set of hollow fiber membranes with a Polyvinylidene difluoride (PVDF) chemistry. The membranes total filtration surface is of 4 m² and havea circulation flow capacity of 40 m3 per hour.

PVDF membrane planned for installation in Langage AD



n-line ultra-filtration membrane- Langage AD system

Ceramic membrane set-up for ultra-filtration

Cleaning procedure

In order to maintain an efficient flow rate of the filtered permeate during ultra-filtration, cleaning procedures should be put in place after the use of the membrane, especially when filtering digestate that has a high solid content and a bacterial content that could potentially damage the filtration membranes.

Here is a detailed procedure for the cleaning of a 0.1 µm pore size ceramic membrane:

- Rinse the system with water until no dirt or particles can be seen (this will take around one hour). Fill the tank with water and turn the pump on – more debris will appear. Empty the tank again and repeat this until minimal amounts of debris can be seen.
- Fill the tank with water once more and add NaOH until the pH reaches 12. Turn the pump on and leave the mix to circulate in the membrane for one hour.
- Empty the system and rinse with water.
- Fill the tank with fresh water and add sulphuric acid until the pH is below 4. Turn the pump on and leave the mix to circulate in the membrane for one hour.
- Empty the system and rinse with water.
- Fill the tank with fresh water and add some detergent, leave for half an hour and rinse thoroughly by filling the tank turning the pump on then off and emptying the system.
- When the system is clean empty the tank and store the ultra-filtration set-up appropriately.

Appendix 7. Digestate Characterisation - Partner Table

Partners	LANGAGE AD	SWANSEA UNIVERSITY (Langage AD digestate)	INNOLAB outsourced AD plant	UBO-CNRS	COOPERL
Digestate Origin	Food Waste and Dairy factory waste	Food Waste and Dairy factory waste	Plant origin and food waste	Pig manure	Pig manure
pH	8.1	7.87	7.87	8.0-8.1	
Dry Matter (%)	**2016**: 5.53; **Feb-2018**: 2.43; **Jun-2018** : 5.94; **Jul-2018**: 5.97	3%	1.23%	0,6-0,9 %	7.62 g/L, 0.78 ± 0.084 %
Digestate pre-treatment	Raw digestate straight out of the digester	Raw digestate delivered from LANGAGE-AD. Dilution and settlement followed by ultrafiltration on 0.1 µm ceramic membrane	Liquid fraction of on-site pre-treated digestate received from Innolab outsourced AD plant. Paper filtration (4-11 µm) of liquid fraction of digestate	Centrifugation (at Cooperl site), pH adjusted to 7, then autoclaved, filtration considered	Centrifugation: Floculation (addition of a polymer) and mechanical decantation. Continuous centrifugation
N concentration (mg/L or mg/Kg)	**2016**: 3161 mg/kg**; Feb-2018**: 3231 mg/kg; **Jun-2018** : 3509 mg/kg; **Jul-2018**: 3550 mg/kg (NH4-N data converted, conversion factor : 0.82243)	**Raw**: 6850 mg/L	**Raw liquid fraction**: Total N: 2430 mg/Kg		TN: 2.48 ± 0.132 g/Kg
		Treated: 4474 mg/L (NH4-N data converted, conversion factor : 0.82243)	**Paper filtered liquid fraction:** Total N: 2370 mg/kg	2300-2650 mg/Kg	**Raw dried digestate** : TN: 320.2 ± 29.945 g/Kg
P concentration (mg/L or mg/Kg)	**2016**: 513 mg/kg; **Feb-2018**: 294 mg/kg; **Jun-2018**: 639 mg/kg; **Jul-2018**: 646 mg/kg	**Raw:** 183 mg/kg	**Raw liquid fraction:** Total P: 25.30 mg/Kg		TP: 0.138 ± 0.052 gP2O5/Kg
		Treated: 135 mg/Kg	**Paper filtered liquid fraction**: Total P: 9.61 mg/kg	25-48 mg/Kg	**Raw dried digestate**: TP: 17.54 ± 4.968 gP2O5/Kg
N:P ratio	**2016**: 6.2; **Feb-2018**: 10.9; **Jun-2018**: 5.5; **Jul-2018**: 5.5	**Raw:** 37.4 ; **Treated:** 33.1	**Raw**: 96.05; **Paper filtration**: 246.62	67.8	**Raw:** 17.9; **raw dried**: 18.2

Partners	LANGAGE AD	SWANSEA UNIVERSITY	INNOLAB outsourced AD plant	UBO-CNRS	COOPERL
Heavy Metals/Trace Elements (mg/Kg)	**2016**: Cd: 0.02; Cr: 0.78; Cu: 3.86; Pb<0.5; Hg<0.05; Ni: 0.45; Zn: 5.93; **Feb-2018**: Cd< 0.05; Cr: 2.2; Cu: 6.6; Pb<0.05; Hg<0.05; Ni<0.05; Zn<0.05 ; **Jun-2018**: Fe: 488; Mo: 0.38; Cu: 2.75; Mn: 4.76; Se: 0.24; Ni: 1.83; Zn: 8.87; Co: 0.25; **Jul-2018**: Fe: 527; Mo: 0.36; Cu: 2.58; Mn: 4.58; Se: 0.21; Ni: 1.65; Zn: 9.84; Co: 0.21	**Raw:** Al: 109.3; Ti: 74.1; Cr: 0.8; Mn: 3.5; Fe: 513.3; Co: 1.7; Ni: 1.4; Cu: 3.1; Zn: 9.5; Se: 0.2; Br: 3.4; Rb: 2.0; Sr: 7.9; Pb: 1.5	**Raw** : Zn: 12.6; Pb: 2.53; Ni: 0.359; Hg: 0.993; Cu: 2.53; Cr: 1.26; Cd: 0.253; As: 2.53		
		Treated: Al: 18.2; Ti: ND; Cr: 0.1; Mn: ND; Fe: 12.2; Co: ND; Ni: 0.3; Cu: 0.6; Zn: 0.9; Se: ND; Br: 3.3; Rb: 1.7; Sr: 0.7; Pb: 0.12	**Paper filtered** : Zn: 1.19; Pb: 0.239; Ni: 0.09; Hg: 0.024; Cu: 0.239; Cr: 0.133; Cd: 0.024; As: 0.239		
Macro Elements (mg/kg)	**2016:** K: 1773; Mg: 126; S: 384; Na: 1496 mg/L; Cl: 3190 mg/L; **Feb-2018**: K: 1604; Mg: 29; S: 135; **Jun-2018**: K: 1910; Ca: 6302; S: 450; **Jul-2018**: K: 1988; Ca: 6310; S: 476	**Raw**: Na: 1150; Mg: 113.7; Si: 189.3; S: 227.3; Cl: 918.3; K: 1363.3; Ca: 1683.3	**Raw**: Ca: 146; Mg: 133; K: 2130		Ca: 188 ± mg/Kg Mg: 26.4 ± mg/Kg Na: 426 ± mg/Kg K: 1054 ± mg/kg
		Treated: Na: 2673.3; Mg: 14; Si: 15.5; S: 18; Cl: 1293.3; K: 1133.3; Ca: 54.8	**Paper filtered**: Ca: 35.8; Mg: 92.1; K: 1720	K: 1250 mg/Kg; Ca:107-129 mg/Kg; Mg: 10-16 mg/Kg; Na:70-470 mg/Kg	**Raw dried digestate** Ca: 23.68 ± g/Kg; Mg: 3.34 ± g/Kg; Na: 53.48 ± g/Kg; K: 217 ± g/kg
Other	Volatile solids: **Feb-2018** : 1.41 % **Jul-2018** : 3.30 % Acetic Acid: **Jun-2018**: 169 mg/L **Jul-2018**: 92 mg/L		**Paper filtered**: dry organic matter: 24.19 % DM; acetic acid: 361 mg/kg; propionic acid: 29 mg/kg; EC: 28.99 mS/cm; redox potential: -417 mV		Organic matter: Raw digestate (RD): 1.82 ± 0.545 g/Kg; Raw dried digestate (RDD): 231.98 ± 47.958 g/Kg; Mineral matter: RD: 0.62 ± 0.084 %; RDD: 76.8 ± 4.779%; Organic carbon: RD: 0.1 %; RDD: 11.6 ± 2.406 %
Analysis methods for N and P	Total Nitrogen: BS EN 13654-1 (Kjeldahl method) or BS EN 13654-52 (Dumas method) Total Phosphorus: BS EN ISO 15587-1:2002	TN: Use of Total Nitrogen and Ammonium Kits-colorimetric methods (Hach 2714100; LCK302; LCK303) and Segmented flow nutrient analyser (AA3-SEAL Analytical). TP: Use of Total Phosphate Kits-colorimetric methods (Hach 2767245) and XR-fluorescence analyser.	Total nitrogen : Kjeldahl method; Total P : ICP-OES	Total nitrogen: Kjeldhal method; Total Phosphorus: ICP-OES	Total nitrogen: Kjeldhal method

Appendix 8. Digestate Characterisation - Literature Table

Reference	Digestate Origin	pH	N concentration (mg/L)	P concentration (mg/L)	N:P ratio	Heavy Metals/Trace Elements	Macro Elements	Others	Digestate Pre-treatment	Analysis methods for N and P
Godos *et al*. (2009). Long-term operation of high rate algal ponds for the bioremediation of piggery wastewaters at high loading rates. Bioresource technology. 100. 4332-4339.	Swine manure	NA	**TN**: 192.5 mg/L ; **NH_4^+-N**: 136 mg/L	NA	NA	NA	NA	COD: 1962 mg/L	Primary treatment consisted of a 0.15 mm rotary screen followed by sedimentation at a residence time of approximately 5 days. This pretreatment reduced the total suspended solid content by approximately 70%.	TKN and TP were analysed according to Standard Methods (Eaton *et al*. 2005).
Wang. L. *et al*. (2010) Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae *Chlorella sp.* Bioresour. Technol. 101. 2623–2628.	Dairy manure	NA	**TN**: 3456 mg/L; **NH_3-N**: 2232	**TP**: 249.7 mg PO_4/L	13.8	NA	NA	COD : 23760 mg/L TS: 6.60% TVS: 5.10%	Dilution/Filtration	Ammonium (NH_4^+–N), total nitrogen (TN) and total phosphorus (TP) were determined for both undigested and digested dairy manures following the Hach DR 5000 Spectrophotometer Manual (Hach, 2008).
Wang. L. *et al*. (2010) Semi-continuous cultivation of *Chlorella vulgaris* for treating undigested and digested dairy manures. Appl. Biochem. Biotechnol. 162. 2324–2332.	Dairy manure	NA	**TN**: 1722 mg/L; **NH_4^+-N**: 1554 mg/L	**TP**: 111.6 mg/L	15.4	NA	NA	COD: 10320 mg/L TS: 6.80% TVS: 5.30%	Dilution	Samples were centrifuged at 5,000 rpm for 15 min and supernatants were collected for the analyses of ammonium (NH_4^+–N), total nitrogen (TN), total phosphorus (TP). Measurements of NH_4^+, TN, TP were performed following the Hach DR 5000 Spectrophotometer Manual
Park. J. *et al*. (2010) Ammonia removal from anaerobic digestion effluent of livestock waste using green alga *Scenedesmus sp.* Bioresour. Technol. 101. 8649–8657	Livestock waste	8.4	**TN**: 1220 mg/L; **NH_4^+-N**: 1196 mg/L	**TP**: 75 mg/L	16.2	NA	NA	COD: 1042 SCOD: 1035 TOC: 195 Alkalinity: 5562 mg/L	Filtration/Autoclave/Dilution	Standard methods (APHA, 1995). HACH DR4000U was used in determination of TN and TP. For NH4-N, HACH DR4000U and Orion electrode were utilized.
Kumar *et al*. (2010). Influence of nutrient loads, feeding frequency and inoculum source on growth of *Chlorella vulgaris* in digested piggery effluent culture medium. Bioresource Technology. 101. 6012-6018	Raw piggery effluent	7.6	**TAN**: 1029.1 mg/L **TKN**: 3304 mg/L, **NO_3^--N** < 2.5 mg/L	**PO_4^{3-}-P**: 192 mg/L	17.2	NA	NA	COD: 12152 mg/L TS: 7.1 g/L VS: 4.4 g/L	Before used as culture media, digested effluent was autoclaved at 120 °C for 15 min	TN determined by Kjeldahl method, TAN was determined by using the Phenate method (APHA, 1992); orthophosphate was determined by using the Merck kit orthophosphate (APHA, 1992)

Reference	Digestate Origin	pH	N concentration (mg/L)	P concentration (mg/L)	N:P ratio	Heavy Metals/Trace Elements	Macro Elements	Others	Digestate Pre-treatment	Analysis methods for N and P
Singh. M. *et al*. (2011) Microalgal system for treatment of effluent from poultry litter anaerobic digestion. Bioresour. Technol. 102. 10841–10848 53.	Poultry litter	NA	Average of 2 batches **TN**: 2021.5 mg/L ; **NH_4^+-N**: 1465 mg/L; **NO_3^--N**: 5.625 mg/L	Average of 2 batches **TP**: 184 mg/L	10.9	Average of 2 batches Al: 20.56; Cd: 0.5445; Cr: 0.6; Cu: 15.9; Fe: 33.85; Pb: 1.63; Mn: 6.97; Mo: 0.93; Ni: 0.765; Zn 9.87 (mg/L)	Average of 2 batches B: 2.23; Ca: 240.5; Mg: 83.75; K: 1866; Si: 49.05; S: 133 (mg/L)	NA	Centrifugation/Dilution	Standard methods (APHA, 2005)
Bchir *et al*. (2011). Optimization of *Spongiochloris sp*. biomass production in the abattoir digestate. Bioresource Technology. 102. 3869-3876.	Abattoir wastewa -ter	7.21	NA	NA	NA	NA	NA	COD: 0.26 g/L DW: 1.07 g/L	The used AD was filtered on Millipore membrane 0.45 µm to eliminate protozoa and other microalgae	NA
Levine. R.B. *et al*. (2011) *Neochloris oleoabundans* grown on anaerobically digested dairy manure for concomitant nutrient removal and biodiesel feedstock production. Biomass Bioenerg. 35. 40–49.	Dairy manure	7.5	**TN**: 3007 mg/L; **NH_4^+-N**: 2097 mg/L; **Organic N**: 910 mg/L	**TP**: 300 mg/L	10	Cu: 9.59 Zn: 20.4 Fe: 64.7 Mn: 16.8 mg/L	K_2O-K: 3262 Ca: 1044 Mg: 659 B: 6.00 mg/L	NA	Dilution	Manure analysis completed by UVM Agricultural & Environmental Laboratory.
Zhou *et al*. (2012). Mass cultivation of microalgae on animal wastewater: A sequential two-stage cultivation process for energy crop and omega-3-rich animal feed production. Applied Biochemestry and biotechnology. 168. 348-363.	Digested swine manure	8.48	**TN**: 4317 mg/L, **NH_3-N**: 3630.1 mg/L	**TP**: 38.9 mg/L	110.9	Fe: 11.66 Al: 1.9 Cu: 1.4 Mn: 0.38 Ni: 0.64 Zn: 4.94 mg/L	B: 2.5 Ca: 99.46 K: 3389.2 Mg: 133.66 Na: 973.5 mg/L	COD: 8933 mg/L TSS: 14.03 g/L TVSS: 9.85 g/L	Large solid particles in the wastewater streams were removed by centrifugation followed by filtration with filter cloth (Wypall X70, Kimberly-Clark Professional). After filtration, the wastewater were autoclaved at 121 °C. Dilution.	Hach protocols (DR 5000 Spectrophotometer)
Franchino. M. *et al*. (2013) Growth of three microalgae strains and nutrient removal from an agro-zootechnical digestate. Chemosphere 92. 738–744.	Cattle slurry and raw cheese whey	7.49	**NH_4^+-N**: 1.634 mg/L	NA	NA	NA	NA	BOD: 20200 COD: 32900 mg/L TVS: 3.1%	Dilution	Nutrient concentrations were determined by using a spectrophotometer LASA 100-HACH LANGE.

Reference	Digestate Origin	pH	N concentration (mg/L)	P concentration (mg/L)	N:P ratio	Heavy Metals/Trace Elements	Macro Elements	Others	Digestate Pre-treatment	Analysis methods for N and P
Cai. T. *et al.* (2013) Cultivation of *Nannochloropsis sauna* using anaerobic digestion effluent as a nutrient source for biofuel production. Appl. Energy 108. 486–492.	Municipal wastewater	NA	**TN**: 2667± 30 mg/L; **TAN**: 2276± 45 mg/L	**TP**: 381± 6 mg/L	7	Al: 1166±43; Fe: 4141±58; Mn: 151.4±19.5; Ni: 25.69±3.64; Co: <0.000; Cu: 26.75±2.39; Zn:105.7±12.3; Mo: 20.93±0.56 ppm	Na: 89±4 K: 121.7±3.5 Ca: 32.95±1.95 Mg: 680±26 ppm	TS: 0.287± 0.005 % ; TVS: 0.208± 0.002 % TC: 2014± 65 COD: 2661± 75 mg/L	Mixed with artificial seawater	The digested sample was diluted 50-fold and analyzed via ICP-MS. TAN, TN, TP were determined using a 3900 spectrophotometer (Hach Company, Düsseldorf, Germany) coupled with a DRB200 dual block reactor (Hach Company, Düsseldorf, Germany)
Abou-Shanab *et al*. (2013). Microalgal species growing on piggery wastewater as a valuable candidate for nutrient removal and biodiesel production. Journal of environmental management. 115. 257-264.	Biologica-lly treated piggery wastewa-ter effluent	8.4	**TN**: 56 mg/L, **NH_4^+-N**: 4.5 mg/L , **NO_3^--N**: 16.8 mg/L	**TP**: 13.5 mg/L , **PO_4-P**: 11.4 mg/L	4.1	Fe: 0.22 Mn: 0.04 Cu: 0.08 Al: 0.04 Cr: 0.03 Ni: 0.03 mg/L	K: 1442 Ca: 84 Mg: 19.7 Na: 409	TC: 571 mg/L TIC: 336 mg/L TOC: 224 mg/L TSS:4.7 mg/L	Microfiltration (0.2 µm)	The total nitrogen, ammonium ions (NH_4^+) and total phosphorus were measured using a Hach Kit (Hach, USA)
Cai *et al*. (2013). Comparison of *Synechocystis sp.* PCC6803 and *Nannochloropsis salina* for lipid production using artificial seawater and nutrients from anaerobic digestion effluent. Biresource Technology. 144. 255-260.	Plant wastes	NA	**TN**: 2667 mg/L, **TAN**: 2276 mg/L	**TP**: 381 mg/L	7	NA	NA	COD: 2661 mg/L	Dilution	HACH 3900 spectrophotometer (Düsseldorf, Germany) coupled with a HACH DRB200 dual block reactor (Düsseldorf, Germany) following the manufacturer's manuals (Hach, 2012a, 2012b, 2012c).
Khanh *et al*. (2013). Selection of microalgae suitable for culturing with digestate from methane fermentation. Environmental Technology. 34. 2039-2045.	Cattle manure	8.4	**TN**: 556 mg/L, **NO_3^--N:** 44 mg/L, **NH_4^+-N**: 866 mg/L	**PO_4^{3-}-P**: 613mg/L	0.9	NA	K: 1120 mg/L Na: 447 mg/L Cl: 417 mg/L SO42-: 332 mg/L	TOC: 286 mg/L	The original digestate was filtered through a 25µm thick membrane filter (0.2 µm diameter pores, Advantec, Japan) to remove large particles and bacteria.	Determined with ion chromatograph (pump: LC-10ADvp, cation column: Shim- pack IC-SC1, anion column: Shim-pack IC-A3, detector: ECD; Shimadzu Co., Japan)
Tan. X. *et al*. (2014) *Chlorella pyrenoidosa* cultivation using anaerobic digested starch processing wastewater in an airlift circulation photobioreactor. Bioresour. Technol. 170. 538–548.	Starch processi-ng wastewa-ter	7.3-7.5	**TN**: 240.3-382.7 **NH_4^+-N**: 217.6-334.7	**TP** : 22.7-40.2 **PO_4^{3-}-P**: 19.3-32.9	9.9	Co: 0.02-0.04 Fe : 0.9-3.6 Cu: 0.09-0.21 Mn: 5.8-8.2 m (mg/L)	B : 0.9-1.5 Ca : 72.8-102.3 K : 102.3-176.4 Mg: 97.6-166.9 (mg/L)	COD : 702.4-1026.2 mg/L	Precipitation/Filtration	The concentrations of COD, NH_4^+-N, PO_4^{3-}-P,TN and TP were measured according to the Chinese State Environmental Protection Agency Standard Methods (SEPA, 2002)

Reference	Digestate Origin	pH	N concentration (mg/L)	P concentration (mg/L)	N:P ratio	Heavy Metals/Trace Elements	Macro Elements	Others	Digestate Pre-treatment	Analysis methods for N and P
Yan. C. and Zheng. Z. (2014) Performance of mixed LED light wavelengths on biogas upgrade and biogas microalga *Chlorella sp.* Appl. Energy 113. 1008–1014.	NA	6.74 ± 0.05	**TN**: 368.29 ± 14.08	**TP**: 10.47 ± 1.38	35.1	NA	NA	COD: 1079.54±31.52 DIC: 1003.82±17.54 DO: 7.25±0.23 (mg/L)	Ultraviolet/Filtration	Standard methods: APHA. Standard methods for the examination of water and wastewater. 19th ed. Washington (DC): American Public Health Association; 1995.
Akerstrom. A.M. *et al*. (2014) Biomass production and nutrient removal by *Chlorella sp* as affected by sludge liquor concentration. J. Environ. Manag. 144. 118–124.	Wastewa -ter sludge	8.1	**TN**: 1210 mg/L; **NH_4^+-N**: 906 mg/L	**TP**: 28mg/L	43.2	Σ (Fe, Mn, Cu, Zn) : 3.7 mg/L Al: 24 mg/L	Σ (K, Ca, S, Mg): 223 mg/L	TSS: 1590 mg/L COD: 3780 mg/L	Mixed with wastewater treatment plant effluent	Hach-Lange kits (Hach Lange, Germany)
Ji. F. *et al*. (2014) Biomass production and nutrients removal by a new microalgae strain *Desmodesmus sp* in anaerobic digestion wastewater. Bioresour. Technol. 161. 200–207	Pig manure	9.18	**TN**: 928.46± 4.64; **NH_4^+-N**: 824.55± 4.20; **NO_3^--N**: 84.46 ±2.86	**TP**: 45.72± 0.55; **PO_4-P**: 39.68 ±0.37	20.3	NA	NA	COD: 6900±53 mg/L	Filtration/Dilution	Total nitrogen (TN) and total phosphorus (TP) were determined colorimetrically as nitrate and phosphate. NH_4–N and PO_4–P were measured following the UV/Vis-spectrophotometric method (National Standard Method of China). The amounts of NO_2–N and NO_3–N were determined with a flow injection analyser (AA3, Seal Analytical).
Uggetti. E. *et al*. (2014) Anaerobic digestate as substrate for microalgae culture: the role of ammonium concentration on the microalgae productivity. Bioresour. Technol. 152. 437–443	Wastewa -ter	NA	**NH_4^+-N**: 950 mg/L	**PO_4^{3-}-P**: 415 mg/L	2.3	NA	Cl: 160 SO42-: 43 Na: 126 K: 240 Mg: 3 Ca: 65 mg/L	COD: 210 mg/L TSS : 1.13 g/L	Dilution	NH_4 –N, NO_2 –N and NO_3 -N were analysed with ion chromatograph (ICS 3000, Dionex, USA).
Cicci and Bravi (2014). Production of the Freshwater Microalgae *Scenedesmus Dimorphus* and *Arthrospira Platensis* by Using Cattle Digestate. Chemical engineering Transactions. 38. 85-90.	Cattle slurry/m-anure and agricultu -ral products	NA	NA	NA	NA	NA	NA	NA	The digestate was sieved at a 710 µm to remove suspended solids and micro-filtered with a poliammide filter (type JX) (pore size 0.3 µm). Thermal sterilisation (20 minutes at 120 °C). Dilution	NA

Reference	Digestate Origin	pH	N concentration (mg/L)	P concentration (mg/L)	N:P ratio	Heavy Metals/Trace Elements		Macro Elements		Others	Digestate Pre-treatment	Analysis methods for N and P
Fouilland *et al*. (2014). Coupling algal biomass production and anaerobic digestion: Production assessment of some native temperate and tropical microalgae. Biomass and Bioenergy. 70. 564-569.	Digested sludge from wastewa -ter treatment plant	NA	**NH_4^+-N**: 1360 mg/L	**PO_4^{3-}-P**: 400 mg/L	3.4	NA		NA		NA	NA	The concentrations of NH_4 and PO_4^3 were determined by ionic chromatography (ICS 3000, Dionex, USA)
Hollinshaead *et al*. (2014). Boosting d-lactate production in engineered cyanobacteria using sterilized anaerobic digestion effluents. Bioresource Technology. 169. 462-467.	Municipa -l waste sludge	NA	**TN**: 208 mg/L	**TP**: 183 mg/L	1.1	NA		NA		COD: 4.5 g/L Butyric acid: 0.02 g/L Propionic acid: 0.49 g/L Acetic acid: 0.41 g/L D-lactate: <0.025 g/L	The AD effluents were filtered then autoclaved before each experiment (stored at 20 °C)	NA
Koreiviene *et al*. (2014). Testing of *Chlorella/Scenedesmus* microalgae consortia for remediation of wastewater, CO 2 mitigation and algae biomass feasibility for lipid production. Journal of environmental engineering and landscape management. 22. 105-114.	Municipa -l waste sludge	7.74	**TN**: 56.5 mg/L	**TP:** 8.3 mg/L	6.8	NA		NA		BOD: 148 mg/L	Wastewater was sterilized by autoclave at 120–130 °C 1 atm. pressure for 30 min. Filtration	standard methods (LST EN ISO 10304; LST EN ISO 14911)
Yang. L. *et al.* (2015) Nutrients removal and lipids production by *Chlorella pyrenoidosa* cultivation using anaerobic digested starch wastewater and alcohol wastewater. Bioresour. Technol. 181. 54–61	Starch processi-ng wastewat er (AW); AD starch wastewat er (ADSW)	AW: 3.7-4.2; ADSW: 7.1-7.3	**TN**: AW:618.68 ± 48.31 ; ADSW: 265.10 ± 19.12 **NH_4^+-N** :AW: 279.72 ± 20.41; ADSW: 240.88 ± 18.89	**TP**: AW:47.16 ± 1.02 ; ADSW: 28.34 ± 1.20	AW: 13.1 , ADSW : 9.4	**AW** Al : 0.21; Fe : 1.47; Zn : 0.10; Mn: 0.57 (mg/L)	**ADSW** Al : 0.12; Fe : 32.86; Zn : 0.86; Mn: 0.13 (mg/L)	**AW** B : 2.45; Ca : 96.14; K : 157.75; Mg: 152.20; Na: 787.74 (mg/L)	**ADSW** B : 4.01; Ca : 98.40; K : 147.47; Mg: 81.16; Na: 719.40 (mg/L)	COD: AW: 65000±1208 ; ADSW: 926.3±65.2 mg/L	Filtration/Sterilisation/Mixed with alcohol wastewater	The concentrations of total phosphorus (TP), orthophosphate (PO_4^{3-}), ammonium (NH_4^+-N), and total nitrogen (TN) were measured according to the Chinese State Environmental Protection Agency Standard Methods

Reference	Digestate Origin	pH	N concentration (mg/L)	P concentration (mg/L)	N:P ratio	Heavy Metals/Trace Elements		Macro Elements		Others	Digestate Pre-treatment	Analysis methods for N and P
Cheng. J. *et al*. (2015) Growth optimisation of microalga mutant at high CO2 concentration to purify undiluted anaerobic digestion effluent of swine manure. Bioresour. Technol. 177. 240–246.	Swine manure and sewage	6.0-6.5	**TN**: 1135 mg/L in raw digestate ; 41.19 mg/L in purified digestate	**TP**: 24.5 mg/L in raw digestate ; 60.19 in purified digestate	46.3	AD effluent Fe: 1.46 Zn: 1.56 Mn: 0.19 Mo: 0.0016 Cu: 0.23 B: 0.93 Pb: 0.031 As: 0.028 Hg: 0.0015 Cd: 0.0005 (mg/L)	Purified Digestate Fe: 1.03 Zn: 0.050 Mn: 0.50 Mo: 0.070 Cu: 0.020 B: 0.50 (mg/L)	AD effluent Na: 312.30 K: 809.25 Ca: 165.91 Mg: 36.97 Cl: 438.2 SO42-: 373.2 (mg/L)	Purified digestate Na: 77.46 K: 75.97 Ca: 6.82 Mg: 7.39 Cl: 29.83 SO42-: 29.34 (mg/L)	NA	Centrifugation/Autoclave	NH_3–N, TN and TP of UADESM were determined using a HACH DR890 spectrophotometer coupled with a HACH DRB200 reactor
Zhao. Y. *et al*. (2015) Performance of three microalgal strains in biogas slurry purification and biogas upgrade in response to various mixed light-emitting diode light wavelengths. Bioresour. Technol. 187. 338–345	Livestock waste	6.84 ± 0.11	**TN**: 308.75 ± 21.51 mg/L	**TP**: 9.93 ± 1.27 mg/L	31.1	NA		NA		COD: 1013.87 ± 29.48 DIC: 939.43 ± 25.36 mg/L	Ultraviolet/Filtration	The filtrates of the cultures were analysed for TN, and TP in accordance with standard methods (APHA, 1995).
Serejo. M.L. *et al*. (2015) Influence of biogas flow rate on biomass composition during the optimization of biogas upgrading in microalgal–bacterial processes. Environ. Sci. Technol. 49. 3228–3236.	Vinasse	7.84± 0.13	**TN**: 71 ± 13 mg/L; **NH_4^+-N**: 56±14 mg/L (in diluted digestate)	**TP**: 3.3±0.9 mg/L (in diluted digestate)	21.5	NA		NA		TOC: 117 ± 17 IC: 142±20 COD: 306 ± 37 TSS: 0.13 ± 0.04	Dilution	TN determined using a shimadzu TOC-VCSH analyzer equipped with a TNM-1 chemiluminescence module. N-NH_4^+ measured using an ammonia electrode Orion Dual Star. P determined spectrophotometrically using the ammonium-molybdate method
Ji. F. *et al*. (2015) Fed-batch cultivation of *Desmodesmus sp* in anaerobic digestion wastewater for improved nutrient removal and biodiesel production. Bioresour. Technol. 184. 116–122.	Pig manure	9.0	**TN**: 774.47 ± 32.99 mg/L **NH_4^+-N**: 708.78 ± 17.17 mg/L ; **NO_3^--N**: 70.12 ± 2.76 mg/L	**PO_4-P**: 31.24 ± 0.56 mg/L	24.7	NA		NA		COD: 4,050 ± 319 mg/L	Filtration/Dilution	Samples were centrifuged and filtered before analysis. The filtrates were properly diluted and analyzed for NH_4-N, PO_4-P and COD concentration according to the spectrophotometric method cited in Hach DR 2700 Spectrophotometer Manual (Hach Company, USA). The amount of NO_3-N was measured with a flow injection nalyzer (AA3, Seal Analytical Inc., UK). TN was determined colorimetrically as nitrate after the samples had been oxidized.

Reference	Digestate Origin	pH	N concentration (mg/L)	P concentration (mg/L)	N:P ratio	Heavy Metals/Trace Elements	Macro Elements	Others	Digestate Pre-treatment	Analysis methods for N and P
Dickinson. K.E. *et al*. (2015) Simultaneous remediation of nutrients from liquid anaerobic digestate and municipal wastewater by the microalga *Scenedesmus sp* AMDD grown in continuous chemostats. J. Appl. Microbiol. 118. 75–83.	Swine manure WW+AD 1.6 and WW+AD 2.4	NA	WW+AD1.6 **NH_3-N** : 3.88±0.16x10-2 g/L **N:P** : 15:1 WW+AD2.4 **NH_3-N**: 5.88±0.18x10-2 g/L **N:P** : 14:1	WW+AD1.6 **PO_4-P** : 5.66±0.81x10-3 g/L WW+AD2.4 **PO_4-P**: 9.55±0.59x10-3 g/L	WW+ AD1.6 : 6.8 ; WW+ AD2.4 : 6.2	NA	NA	TS: WW+AD1.6 5.08±0.01 g/L ; WW+AD2.4 1.20±0.1 g/L	Autoclave/Mixed with municipal wastewater. Chemostat vessels were initially set up and autoclaved with municipal wastewater and digestate. All inflow municipal wastewater was sterilized by passage through a tangential flow membrane filtration system. Digestate was autoclaved for 20 min. at 121°C, cooled and added to the wastewater.	Dissolved ammonium, phosphate, total nitrogen and total Kjeldahl nitrogen (TKN) concentrations of the inflow media and residual free dissolved inorganic ammonium and phosphate concentrations of the cultures were determined using commercially available, colorimetric assay systems using a DR 2800 portable spectrophotometer (TNT831/832, TNT843 and TNT880; Hach Co., Loveland CO).
Xu. J. *et al*. (2015) Nutrient removal and biogas upgrading by integrating freshwater algae cultivation with piggery anaerobic digestate liquid treatment. Appl. Microbiol. Biotechnol. 99. 6493–6501	Livestock waste	6.43±0.09	**TN**: 120.69±9.65 mg/L	**TP**: 129.22±9.16 mg/L	0.9	NA	NA	COD: 3200.26±39.81 TS: 304.67±19.44 mg/L	Filtration/Autoclave/Dilution Pre-treatment was performed via sedimentation and filtration using a filter cloth to remove large non-soluble particulate solids. Autoclaved for 20 min at 121 °C, and liquid stored at 4 °C for 2 days for any visible particulate solids to settle, and supernatant was used to study the microalgae growth.	The filtrates of the cultures were analysed for TN, and TP using standard methods (APHA 1995)
Lu *et al*. (2015). Cultivation of *Chlorella sp.* using raw diary wastewater for nutrient removal and biodiesel production: Characteristics comparison of indoor bench-scale and outdoor pilot-scale cultures. Bioresource Technology. 192. 382-388.	Dairy farm effluent (RDW)	8.18 ± 0.03	**TN**: 283.00 ± 12.73 ; **NH_4^+-N:** 181.50 ± 9.19 mg/L	**TP**: 115.90 ± 7.50 mg/L	2.4	NA	NA	Suspended solids: 1.74 ± 0.09 mg/L COD: 2593.00 ± 15.56 mg/L	Leave to settle overnight and filtered through gauze	Centrifugation at 5000 rpm for 5 min. filtration on a 0.22 µm nylon membrane filter, after which the filtrates were appropriately diluted. Finally, COD, ammonium, TN and TP concentration of the filtrates were measured following the Hach DR2700 Spectrophotometer Manual (Hach, 2008).
Veronesi *et al*. (2015). Microalgae Cultivation : Nutrient Recovery from Digestate for Producing Algae Biomass. Chemical engineering transactions. 43. 1201-1206	NA	8.68	**TKN**: 1488 mg/Kg, **NH_4^+-N**: 1435 mg/Kg	**TP**: 31.3 mg/Kg	47.5	NA	NA	TS: 8.9 g/Kg	Dilution	TKN and N-NH_4^+ determined using fresh material according to the analytical methods for wastewater sludges (IRSA CNR, 1994). Total phosphorus was determined by means of inductively coupled plasma atomic emission spectroscopy (ICP-MS, Varian, Fort Collins,USA).

Reference	Digestate Origin	pH	N concentration (mg/L)	P concentration (mg/L)	N:P ratio	Heavy Metals/Trace Elements	Macro Elements	Others	Digestate Pre-treatment	Analysis methods for N and P
Olguín *et al*. (2015). Anaerobic digestates from vinasse promote growth and lipid enrichment in *Neochloris oleoabundans* cultures. Journal of Applied Phycology. 27. 1813-1822.	Digested vinasse	8.1	**TN**: 579.6 mg/L, **NH_4^+-N**: 473.3 mg/L	**TP**: 98.6 mg/L	5.9	NA	NA	COD: 7944 mg/L Sulfates: 1331.3 mg/L VFA: 697.1 mg/L	Anaerobic effluents from vinasse (AEV) were left to settle for 4 h, and the liquid fraction was collected daily, filtered with a polyester membrane with a 45-µm pore size, and refrigerated (5 °C) until use. Dilution.	Colorimetric method (Nessler) using a HACH DR4000 spectrophotometer
Markou (2015). Fed-batch cultivation of *Arthrospira* and *Chlorella* in ammonia-rich wastewater: Optimization of nutrient removal and biomass production. Bioresource Technology. 193. 35-41.	Poultry litter	7.85	**NH_4^+-N**: 4315 mg/L	**PO_4-P**: 83 mg/L	51.9	Fe: 4.33 mg/L Mn: 471 µg/L Zn: 134 µg/L Ni: 421 µg/L Cu: 585 µg/L Pb: 150 µg/L Cd > 3 µg/L	K: 2590 mg/L Na: 261 mg/L Mg: 10.43 mg/L	COD: 25821 mg/L TDS: 4.53 g/L VFA: 13958 mg/L Carbohydrates: 310 mg/L Proteins: 4879 mg/L	The effluents (liquor) of the anaerobic digestion were centrifuged at 5000 rpm for 10 min and the supernatant was used for the experiments	Flame atomic absorption spectrometry (Varian AA-200). Potassium was measured by flame-photometer (Sherwood Scientific, model 400)
Marcilhac *et al*. (2015). Control of nitrogen behaviour by phosphate concentration during microalgal-bacterial cultivation using digestate. Bioresource technology. 175. 224-230	Digestate from wastewa -ter treatmen t plant	NA	In produced growth medium: **$NH4_+^-$N**: 190 mg/L	**TP:** 67, 23, 8 and 3 mg/L	2.8, 8.3, 23.7 and 63.3	NA	NA	NA	Centrifugation and Dilution	NA
Wahal & Viamajala (2016) Uptake of inorganic and organic nutrient species during cultivation of a *Chlorella* isolate in anaerobically digested dairy waste. Biotechnology progress. 32. 1336-1342.	Dairy waste	7.0-7.2	**Digestate diluted 20x TN**: 349.8 mg/L ; **NH_4^+-N**: 206.9 mg/L; **org-N**: 142.9 mg/L	**Digestate diluted 20x TP**: 46.4 mg/L ; **PO_4^{3-}-P**: 31.6 mg/L; **org-P**: 14.8 mg/L	7.5	NA	NA	Digestate diluted 20x COD: 3850 mg/L	the samples were diluted fourfold with DI water to (partially) solubilize mineral precipitates and centrifuged at 500g for 5 min to remove heavy and insoluble particulates and stored at 8°C.	Samples were filtered through 0.45 lm syringe filters and analyzed for dissolved ammonium nitrogen (NH_4-N), total nitrogen (TN) (Standard Method 4500N-C), dissolved total phosphorous (TP) (Standard Method 4500P-I), ortho phosphorus (ortho-P) (Standard Method 4500P-E).
Choudary *et al*. (2016). Screening native microalgal consortia for biomass production and nutrient removal from rural wastewaters for bioenergy applications. Ecological Engineering. 91. 221-230.	Livestock waste	7.8±0.1 1	**TAN**: 161±0.57 mg/L, **NO_3^--N**: 75±1.52 mg/L	**TP**: 200±2 mg/L	0.8	NA	NA	COD: 2940±1.48 mg/L TDS: 4480±29 mg/L TSS: 120±5.26 mg/L	The large solid particles were removed from wastewater by sedimentation followed by filtration using muslin cloth (pore size ≈0.5–1.5 mm)	Standard methods (Eaton *et al*., 2005) or Hach Protocols

Reference	Digestate Origin	pH	N concentration (mg/L)	P concentration (mg/L)	N:P ratio	Heavy Metals/Trace Elements	Macro Elements	Others	Digestate Pre-treatment	Analysis methods for N and P
Ledda *et al*. (2016). A simplified process of swine slurry treatment by primary filtration and *Haematococcus pluvialis* culture to produce low cost astaxanthin. Ecological Engineering. 90. 244-250.	Pig waste	pre-treatment: 8.56, post-treatment: 7.98	pre-treatment: **TN**: 543 mg/L, **NH_4^+-N**: 226 mg/L, **NO_3^--N**: 51.9 mg/L; post-treatment: **TN**: 272 mg/L, **NH_4^+-N**: 40 mg/L, **NO_3^--N**: 235 mg/L	pre-treatment: **TP**: 25.8 mg/L; post-treatment: **TP**: 22.5 mg/L	Pre-treatment: 21.04; post-treatment: 12.1	Post treatment only: Fe: 4.4 Zn: 1.4 Cu: 0.2 Mn: 0.5 mg/L	Post treatment only: K: 454 Mg: 28.4 Na: 131 Ca: 95 mg/L	Pre-treatment: COD: 2160 mg/L, post-treatment: COD: 812 mg/L	Filtration as downstrean system when sampled from digester and extra filtration pre-algal culture : 0.45 Whatman GF/C filters	Inductively coupled plasma mass spectrometry (ICP-MS, Varian, Fort Collins, USA) according to 3051A and 6020A EPA methods (EPA, 2007a,b).
Ledda *et al*. (2016). Integration of microalgae production with anaerobic digestion of dairy cattle manure: An overall mass and energy balance of the process. Journal of cleaner production. 112. 103-112	Cattle manure	7.95	**TKN**: 1211 mg/L, **NH_4^+-N**: 1130 mg/L	**TP**: 17 mg/L	71.2	NA	NA	TS: 7.2 % VS: 75.1 %	Liquid fraction is treated by the addition of a polyamide flocculant and sent to a decanter centrifuge. Centrifuged liquid enters the ultrafiltration unit, equipped with a 40 kDa membrane. Permeate can be used to produce microalgae, but can also be subjected to reverse osmosis. Permeate from reverse osmosis is refined in a zeolites bed and then discharged to surface-water bodies. The concentrate from reverse osmosis enters a cold ammonia stripping unit where lime is added, raising pH up to 12-12.5	TKN and ammonia nitrogen ($N\text{-}NH_4^+$) were determined using fresh material according to the analytical methods for wastewater sludge (IRSA CNR, 1994). Total phosphorus (P) content was determined by inductively coupled plasma mass spectrometry (ICP-MS, Varian, Fort Collins, USA)
Franchino *et al*. (2016). Microalgae treatment removes nutrients and reduces ecotoxicity of diluted piggery digestate. Science of the total environment. 569-570. 40-45.	Pig slurry and corn	8.0	**TN**: 3355 mg/L, **NH_4^+-N**: 2050 mg/L, **NO_3^--N**: 229.5 mg/L	**PO_4^{3-}-P**: 318.5 mg/L	10.5	NA	NA	COD: 17600 mg/L	Dilution	APAT-IRSA CNR standard methods (2003)
Wen *et al*. (2017) Isolation of an indigenous Chlorella vulgaris from swine wastewater and characterization of its nutrient removal ability in undiluted sewage. Bioresour. Technol. 243. 247–253	Swine wastewater from local pig farm	8.36	**TN**: 313.25 ± 19.35 mg/L	**TP**: 56.18 ± 12.44 mg/L	5.6	Zn <0.05 Mn <0.01 Cu < 0.05 mg/L	Cl: 392.42 ± 33.85 K: 381.45 ± 22.58 Ca: 23.77 ± 7.29 Mg: 7.06 ± 0.53 mg/L	COD: 796.21 ± 24.67 mg/L	The original swine wastewater was filtered through a triple gauze layer to remove insoluble solids. After filtration, the wastewater was autoclaved at 121 °C for 20 min	Chinese National Standards (Cheng *et al*., 2007; Jin *et al*., 2015

Reference	Digestate Origin	pH	N concentration (mg/L)	P concentration (mg/L)	N:P ratio	Heavy Metals/Trace Elements	Macro Elements	Others	Digestate Pre-treatment	Analysis methods for N and P
Silkina *et al*. (2017) Formulation and utilisation of spent anaerobic digestate fluids for the growth and product formation of single cell algal cultures in heterotrophic and autotrophic conditions. Bioresour. Technol. 244. 1445-1455.	mixed waste of cattle slurry, vegetable waste and silage	8.38	**NH_4^+-N**: Raw Sludge: 2.21 ; N:P (16.53): 0.69 g/L; N:P (3.78): 0.27g/L ; N:P (14.22): 0.36 g/L	**TP**: Raw Sludge: 2.03 ; N:P (16.53): 0.04; N:P (3.78): 0.07 ; N:P (14.22): 0.03 g/L	16.53, 3.78 and 14.22	(Ca, Cu, Co, Fe, Pb, Mg, Mn, Zn, K, As) : Raw Sludge: 3.10 ; N:P (16.53): 0.88; N:P (3.78): 0.68 ; N:P (14.22): 0.59 g/L	NA	TSS: 1.71 g/L TDS: 11.54 g/L TS: 27.21 g/L	Microfiltration through a pilot scale unit equipped with a ceramic membrane (Pall Membralox, 3.70 mm channels, 0.22 m2 area with nominal pore size <0.2 lm). To achieve varying concentrations of nutrients, a scheme combining diafiltration with and without pre-acidification was applied.	NA
Labbé *et al* (2017) Microalgae growth in polluted effluents from the dairy industry for biomass production and phytoremediation. Journal of environmental chemical engineering. 5. 635-643.	Dairy industry: Cattle standing yard effluent (CSYE)	7.82	**NH_4^+-N**: 28.4 mg/L,**NO_3^--N**: 14.8 mg/L	**TP**: 5.1 mg/L	5.6	Fe: 0.57 Mn: 0.23 Zn: 0.1 Cu: 0.03 B: 1.5 As: < 0.01 Pb: 0.04 Cd: <0.01 mg/L	K: 78 Ca: 174 Mg: 43 SO42-: 384 Na: 136 HCO3-: 458 Cl-: 163 mg/L	Dissolved Oxygen: 13.27 mg/L	Washed with water. Settled for 24 h and filtered through 1 mm mesh size prior to use in the experiment in order to remove settled and large solids	Culture media composition was determined in a certified laboratory. NO_3^- and NH_4^+ were determined by potentiometry
Hajar *et al*. (2017) Cultivation of *Scenedesmus dimorphus* using anaerobic digestate as a nutrient medium. Bioprocess Biosystem engineering. 40. 1197-1207.	Food waste and animal manure	NA	**2.5% dilution TN**: 109 mg/L, **NH_4^+-N**: 78.8 mg/L **1.25% dilution TN**: 55 mg/L, **NH_4^+-N**: 39.2 mg/L	**2.5% dilution TP**: 10.5 mg/L **1.25% dilution TP**: 4.5 mg/L	2.5% dilution: 10.4 ; 1.25% dilution: 12.2	NA	NA	2.5% dilution COD: 498 mg/L TSS: 61.6 mg/L 1.5% dilution COD: 277.5 mg/L TSS: 48.5 mg/L	Dilution	Colorimetric methods in compliance with APHA Standard Methods for the Examination of Water and Wastewater and EPA methods (HACH methods 10072, 10127, and 10031, and 8000) using HACH DR 3900 spectrophotometer.
Tao *et al*. (2017). Cultivation of *Scenedesmus acuminatus* in different liquid digestate from anaerobic digestion of pulp and paper industry biosludge. Bioresource Technology. 245. 706-713.	pulp and paper industry biosludge	8.0	**NH_4^+-N**: 380±20, **NO_3^--**: <1.0, **NO_2^--**: < 1.0 mg/L	**TP**: 33±3 mg/L, **PO_4^{3-}-P**: 16±3 mg/L	11.5	NA	NA	COD: 1200±130 mg/L DOC: 300±4 mg/L DIC: 570±10 mg/L	Digestate was centrifuged at 5200 rpm for 4 minutes and the supernatant was filtered through a glass fiber filter (Whatman GF/A, UK). Stored at 4°C.	NH4+-N was measured with an ion-selective electrode (Thermo Scientific Orion ISE meter). NO_3^-,NO_2^-,PO_4^{3-}, were measured using an ICS-1600 ion chromatograph (Dionex, USA) with an AS-DV autosampler, Ion-Pac AS4A-SC anion exchange column, and ASRS-300 suppressor (2 mm).

Reference	Digestate Origin	pH	N concentration (mg/L)	P concentration (mg/L)	N:P ratio	Heavy Metals/Trace Elements	Macro Elements	Others	Digestate Pre-treatment	Analysis methods for N and P
Massa *et al*. (2017). Evaluation of anaerobic digestates from different feedstocks as growth media for *Tetradesmus obliquus, Botryococcus braunii, Phaeodactylum tricornutum* and *Arthrospira maxima*. New Biotechnology. 36. 8-16.	Zootechnical digestate (ZWLD); Vegetable digestate (VWLD); municipal solid waste (MW LD)	NA	**ZWLD: NH_4^+-N**: 1400 mg/L, **NO_3^--N**: 230 mg/L **VWLD: NH_4^+-N**: 2000 mg/L, **NO_3^--N:** 890 mg/L **MWLD: NH_4^+-N:** 2650 mg/L, **NO_3^--N:** 720 mg/L	**ZWLD: PO_4-P**: 716 mg/L, **VWLD: PO_4-P**: 66 mg/L, **MWLD: PO_4-P:** 24 mg/L,	ZWLD: 1.9 ; VWLD: 30.3 ; MWLD: 110.4	NA	NA	ZWLD: TS: 23.4 g/L, COD: 14100 mg/L VWLD: TS: 20.5 g/L, COD: 22120 mg/L MWLD: TS: 52.8 g/L, COD: 19800 mg/L	Digestate was filtered progressively from 250 to 50 mm and centrifuged at 2,500g for 20 min. Autoclaved to inactivate any contaminating organisms. Sterilization of the digestates by autoclaving showed a decrease in ammonia concentration of about 50–60%.	Spectrophotometric test kits (Hach-Milano)
Salati *et al*. (2017). Mixotrophic cultivation of *Chlorella* for local protein production using agro-food by-products. Bioresource Technology. 230. 82-89	Cheese Wey (CW)	5.26	**TN**: 805 ± 48 mg/L ; **NH_3-N**: 103 ± 9 mg/L	**TP**: 400 ± 20 mg/L	2	NA	NA	DM: 158±6 g/kg; TOC: 55.1±1.6 g/L; COD: 147±7 mg/L	De-proteinization that was performed by using heat treatment at 115 °C for 15 min, Filtration of the flocs formed by using a 0.2 µm Whatman filter. Enzymatic hydrolysis of CW was performed by using b-galactosidase (13.5 units mg, Sigma–Aldrich, San Luis, Missouri, USA) at 30 °C and pH 4.5, for 24 h in a shake flask at 200 rpm using 65 U of enzyme per g lactose quantified in whey permeate	Total Kjeldahl Nitrogen (TKN) was determined according to standard methods (APHA, AWWA, WEF, 2005). Total phosphorus (TP) was determined by inductively coupled plasma mass spectrometry (ICP-MS, Varian, Fort Collins, USA) according to the 3051A and 6020A EPA methods (EPA, 2007)
Huy *et al*. (2018). Photoautotrophic cultivation of mixed microalgae consortia using various organic waste streams towards remediation and resource recovery. Bioresource technology. 247. 576-581	Animal manure (AM) and digested sludge (DS)	AM: 8.83 DS: 8.84	AM: **TN**: 323 mg/L DS: **TN**: 746.5 mg/L	AM: **TP**: 21 mg/L DS: **TP**: 55 mg/L	AM: 15.3; DS: 13.6	NA	NA	TS: AM: 27.92 g/L ; DS: 14.68 g/L VS: AM: 16.18 g/L; DS: 13.04 g/L COD: AM: 18.45 g/L; DS: 16.85 g/L	Dilution	Total Nitrogen and Total Phosphorous were measured by using Humas test kit with a spectrophotometer (Humas, HS3300, Republic of Korea)