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Valorising nutrient-rich digestate: Dilution, settlement and membrane filtration processing for optimisation as a waste-based media for microalgal cultivation

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ABSTRACT

Digestate produced from the anaerobic digestion of food and farm waste is primarily returned to land as a biofertiliser for crops, with its potential to generate value through alternative processing methods at present under explored. In this work, valorisation of a digestate resulting from the treatment of kitchen and food waste was investigated, using dilution, settlement and membrane processing technology. Processed digestate was subsequently tested as a nutrient source for the cultivation of *Chlorella vulgaris*, up to pilot-scale (800L). Dilution of digestate down to 2.5% increased settlement rate and induced release of valuable compounds for fertiliser usage such as nitrogen and phosphorus. Settlement, as a partial processing of digestate offered a physical separation of liquid and solid fractions at a low cost. Membrane filtration demonstrated efficient segregation of nutrients, with micro-filtration recovering 92.38% of phosphorus and the combination of micro-filtration, ultra-filtration, and nano-filtration recovering a total of 94.35% of nitrogen from digestate. Nano-filtered and micro-filtered digestates at low concentrations were suitable substrates to support growth of *Chlorella vulgaris*. At pilot-scale, the microalgae grew successfully for 28 days with a maximum growth rate of 0.62 day⁻¹ and dry weight of 0.86 g·L⁻¹. Decline in culture growth beyond 28 days was presumably linked to ammonium and heavy metal accumulation in the cultivation medium. Processed digestate provided a suitable nutrient source for successful microalgal cultivation at pilot-scale, evidencing potential to convert excess nutrients into biomass, generating value from excess digestate and providing additional markets to the anaerobic digestion sector.

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1. Introduction

Anaerobic digestion (AD) is commonly used in Europe for the treatment of food and farm waste. The AD process is a biological mechanism during which bacterial and archaeal communities convert carbon-rich organic waste into biogases, primarily methane and carbon dioxide (Doble and Kumar, 2005). Another by-product of the AD process is a nutrient-rich digestate (NRD). NRD is rich in carbon, nitrogen (N), phosphorus (P) and other macro and micronutrients (Papadimitriou et al, 2008; Tambone et al, 2017). NRD is primarily used as organic fertiliser and is directly applied onto farmland (Fuchs and Drog, 2013). However, the use of digestate as a soil fertiliser increases the risk of nutrient runoff

and penetration of groundwater resources, leading to soil and water eutrophication (Guilayn et al., 2019). Consequently, Nitrate Vulnerable Zones (NVZs) have been designated under the European Nitrate Directive 91/676/CEE that limits the annual load of nitrogen applied onto farmland. NVZs are on the increase across North West Europe, resulting in the accumulation of approximately 10 million tons of excess digestate (Fuchs and Drog, 2013).

Alternatives to farmland spreading have been investigated, such as using solid digestate for energy production or conversion into added-value products (char or activated carbon) (Monlau et al., 2015), however valorisation of digestate has been underexplored and solutions have yet to be firmly established to create value from this excess NRD. The present study focused on mechanical and biological treatments of digestate to increase its value and marketability. The partial processing of digestate was investigated first, by establishing methods for the separation of liquid and solid fractions of digestate using simple low-cost techniques. This approach

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is known to reduce the volume of digestate, hence minimising the processing cost, and facilitating the transportation of digestate to other locations less impacted by soil eutrophication (Guilayn et al., 2019). On the other hand, complete processing of digestate results in a clear liquor, directly dischargeable into the environment, however this process involves more complex and costly separation techniques such as centrifugation. Ultimately, both partial and complete processing of digestate result in nutrient segregation (e.g. P in the solid fraction and N in the liquid fraction (Tambone et al., 2017)), which allows for the exploitation of digestate, for example in fertiliser formulation, due to the targeting of specific compounds.

Membrane filtration as a technology for the complete processing of digestate has generated a lot of interest in recent years. This technique allows for the removal of particles and microorganisms (e.g. protozoa, bacteria) potentially present in digestate (Ledda et al., 2016; Massa et al., 2017; Mayhead et al., 2018; Park et al., 2010; Wang et al., 2010; Wen et al., 2017). Membrane technology is also used as a means to recover and concentrate nutrients from digestate (Abou-Shanab et al., 2013; Gerardo et al., 2015; Khan and Nordberg, 2018; Olguín et al., 2015; Silkina et al., 2017; Zacharof et al., 2019). During the process, particulates remain in the retentate (i.e. fraction of liquid not going through the membrane and resulting in a thick sludge following concentration); while the liquid nutrient-rich fraction permeates the membrane.

The complete processing of digestate using membrane filtration can have significant upfront costs, especially for large-scale applications. There is subsequently scope to utilise excess digestate and consequent nutrients, for example by conversion into added-value biomass for new markets. One of these novel methods includes the use of micro and macro nutrients present in NRD for microalgal cultivation (Fathi et al., 2013; Judd et al., 2015; Luo et al., 2017; Silkina et al., 2019). The majority of the compounds of interest found in NRD and useful for microalgal production are bound to the solid fraction (e.g. phosphorus), which limits their bioavailability and requires digestate processing for use as a waste-based medium for microalgal cultivation. Coupling the partial and complete processing of digestate to its biological treatment via microalgal production could be a promising technology, increasing digestate value and recouping the cost of heavy processing methods currently used. Furthermore, microalgae production is a promising technology, allowing for large scale applications (Stiles et al., 2018).

The aim of this work was to investigate routes for the valorisation of excess digestate. To do so, a series of digestate treatments were investigated, namely dilution combined with settlement, and membrane filtration using a range of pore sizes. The resulting processed NRD was used as a nutrient source for the cultivation of *Chlorella vulgaris*, a species widely recognised in algal wastewater remediation (Judd et al., 2015). *C. vulgaris* was cultivated up to pilot-scale (800L), with the objective to explore the microalgae potential for converting excess nutrients from NRD into biomass.

2. Materials and methods

2.1. Nutrient-rich digestate

Raw NRD (i.e. sampled directly from the digester tank and not modified) from the industrial anaerobic digestion of kitchen food waste was sourced from the Langage-AD facility located in Plymouth (Devon, UK). The composition of the collected NRD was stable throughout the year, demonstrating a robust anaerobic digestion process. The NRD was transported to Swansea University and stored at 4 °C, to avoid bacterial development. A 500 mL aliquot

of NRD was taken to measure initial pH, dry weight, ammonium (NH₄-N), P and heavy metal composition (Table 2).

NRD was first treated using dilution and settlement and the unsettled layer of NRD was then processed using membrane filtration at a different range of molecular weight cut-offs (MWCO), namely micro-filtration (MWCO: 0.2 µm), ultra-filtration (MWCO: 10 kDa) and nano-filtration (MWCO: 500 Da). The permeates from nano-filtration and micro-filtration were used for the cultivation of *C. vulgaris*.

2.2. Dilution and settlement – Partial processing of digestate

2.2.1. Experimental design and sample collection

Raw NRD was diluted in triplicate to 2.5%, 5%, 10% and 20% with deionised water in 500 mL glass cylinders. Sedimentation rate was measured for 24 h. Conditions were compared against a raw NRD (i.e. undiluted) control tested in quadruplicate. The sedimentation rate of NRD was calculated by measuring the height (in cm) of the first visible separation layer of NRD in the glass cylinders. Measurements were taken every hour for the first five hours of the experiment and for the last two hours of the experiment. After 24 h, samples were collected in layers of 100 mL using a sterile syringe connected to a sterile tube, and samples were stored at 4 °C before analysis. Five layers were collected for each cylinder, defined as Layer 1 (from the top; L1), Layer 2 (L2), Layer 3 (L3), Layer 4 (L4) and Layer 5 (at the bottom of the cylinder; L5).

2.2.2. Dry weight, turbidity and particles distribution analysis

Dry weight (in g·L⁻¹) was measured by filtering 5 to 60 mL samples (the volume was dependent on the dilution factor) using pre-dried and pre-weighed filters (Whatman 47 mm GF/C glass micro-fiber filters, pore size: 1.2 µm, method based on Silkina et al. (2019)). Samples were oven dried for 24 h at 80 °C and dry weight was calculated as the weight difference between the dried-filtered sample and the pre-weighed-filter in relation to the volume of sample filtered (Eq. (1)).

$$dw(g.L^{-1}) = ((fs - f)/Vs) * 1000 \quad (1)$$

Where *dw* is the dry weight in g·L⁻¹, *fs* is the weight of the filtered and dried sample (g), *f* is the weight of the pre-dried filter (g) and *Vs* is the volume of sample filtered (mL).

Sample turbidity was determined using absorbance measured at a wavelength of 750 nm using a spectrophotometer (SPECTROstart^{Nano}, BMG Labtech). Particle distribution analysis was performed using a coulter-counter (Beckman) according to the method described in Mayers et al. (2013). Particle numbers in the range of 1–20 µm were assessed.

2.2.3. Nutrients and elemental composition

For each sampled layer, the NH₄-N concentration was measured using an ammonium reagent kit (Spectroquant[®]), based on the colorimetric quantification of NH₄-N (method analogous to EPA 350.1, APHA 4500-NH3 F, ISO 7150-1, and DIN 38406-5). The absorbance of treated samples was measured at 690 nm according to supplier instructions and measured against a calibration curve to determine NH₄-N concentration. P was measured using a reagent test kits (Spectroquant[®]), also based on colorimetric reactions (method analogous to EPA 365.2 + 3, APHA 4500-P E, and DIN EN ISO 6878). Absorbance was recorded at 410 nm and P concentration was assessed as for ammonium.

Elemental analysis of collected samples was completed using an X-ray fluorescence equipment (XRF, Rigaku Nex CG). XRF is a technique which allows distinction between atoms, based on their X-ray fluorescence spectra: electrons from a sample are excited by the X-ray and emit a fluorescence radiation characteristic to a particular material (Shapovalov et al., 2007). 200 µL of each sample

Table 1

Elemental Analysis (in mg.kg⁻¹) of diluted digestate at concentrations of 2.5%, 5%, 10% and 20% compared to a raw undiluted digestate for five layers collected after 24 h of experiment. Highest concentrations for each element are highlighted in bold.

Treatment	Layers	Na mg.kg ⁻¹	Mg mg.kg ⁻¹	Al mg.kg ⁻¹	S mg.kg ⁻¹	Cl mg.kg ⁻¹	K mg.kg ⁻¹	Ca mg.kg ⁻¹	Mn mg.kg ⁻¹	Fe mg.kg ⁻¹	Cu mg.kg ⁻¹	Zn mg.kg ⁻¹
Control	L1	1213.3 ± 82.2	15.9 ± 0.5	36.6 ± 2.5	62.4 ± 5.2	898 ± 61.7	951 ± 73.8	176.3 ± 11.9	0.84 ± 0.0	68.6 ± 5.8	0.8 ± 0.0	9.1 ± 0.8
	L2	1286.7 ± 4.7	15.9 ± 2	39.3 ± 1.3	70.1 ± 1.7	951.3 ± 11.4	1007.3 ± 24.2	201.7 ± 9.8	0.94 ± 0.1	79.1 ± 4.5	0.92 ± 0.1	10.5 ± 0.4
	L3	1333.3 ± 47.8	17.5 ± 0.7	38.4 ± 1.3	66.2 ± 2.6	940 ± 32.8	987.7 ± 23.2	188.3 ± 8.7	1.04 ± 0.1	73.7 ± 3.0	0.81 ± 0.1	9.7 ± 0.3
	L4	1330 ± 69.8	19.7 ± 1.5	39.2 ± 1.1	69.3 ± 2.4	955.3 ± 18.8	1016.7 ± 17.0	204.3 ± 2.1	1.03 ± 0.1	80 ± 2.3.0	0.91 ± 0.1	10.2 ± 0.3
	L5	1326.7 ± 60.2	30.9 ± 8.3	52.8 ± 6.9	96.5 ± 14.1	1073.3 ± 57.9	1083.3 ± 45.0	590.7 ± 239.1	1.25 ± 0.2	128.7 ± 26.0	0.98 ± 0.2	12.9 ± 1.6
20%	L1	745.5 ± 1.5	12.5 ± 1.0	16.8 ± 0.2	19.7 ± 0.2	584 ± 9.0	466.5 ± 0.5	49.2 ± 0.9	0.22 ± 0.0	16.6 ± 0.4	0.71 ± 0.1	2.9 ± 0.0
	L2	601.5 ± 32.5	9.8 ± 0.6	15.2 ± 0.4	16.5 ± 0.0	561 ± 18.0	443 ± 8.0	47.3 ± 0.0	0.29 ± 0.0	16.4 ± 0.2	0.52 ± 0.1	2.8 ± 0.1
	L3	561 ± 30.0	9.2 ± 1.4	15.4 ± 0.3	16.1 ± 0.6	547.5 ± 13.5	452 ± 12.0	49.8 ± 2.5	0.23 ± 0.0	16.5 ± 0.8	0.81 ± 0.0	2.9 ± 0.2
	L4	629.5 ± 10.5	10.1 ± 1.0	16.4 ± 1.0	17.9 ± 0.5	579 ± 18.0	462.5 ± 6.5	49.4 ± 2.1	ND	16.4 ± 0.5	0.67 ± 0.0	3 ± 0.3
	L5	623.5 ± 5.5	41.1 ± 6.0	25.7 ± 1.4	30.2 ± 2.3	527 ± 33.0	437 ± 16.0	177.5 ± 47.5	0.64 ± 0.0	37 ± 2.4.0	0.64 ± 0.1	2.9 ± 0.4
10%	L1	254.5 ± 23.5	6.5 ± 0.5	8.5 ± 0.1	14.3 ± 0.8	276 ± 11.0	223.5 ± 10.5	41 ± 1.9	ND	9.2 ± 0.3	0.74 ± 0.0	1.9 ± 0.1
	L2	239 ± 22.0	6.4 ± 0.2	8.2 ± 0.4	13.3 ± 0.2	268.5 ± 9.5	220 ± 9.0	40.5 ± 1.5	ND	8.8 ± 0.1	0.75 ± 0.1	1.9 ± 0.0
	L3	250.5 ± 15.5	6.9 ± 0.7	7.9 ± 0.4	13.4 ± 0.4	266.5 ± 9.5	219.5 ± 9.5	40.3 ± 1.3	ND	8.8 ± 0.3	0.71 ± 0.0	2.1 ± 0.0
	L4	240 ± 10.0	5.5 ± 0.1	8 ± 0.0	13 ± 0.1	268.5 ± 0.5	215.5 ± 6.5	39.2 ± 0.4	ND	9 ± 1.0	0.87 ± 0.0	2.2 ± 0.1
	L5	284.5 ± 10.5	17.7 ± 7.1	11.3 ± 1.9	17.2 ± 1.3	280 ± 11.0	228 ± 7.0	69.4 ± 26.4	ND	12.9 ± 2.9	0.75 ± 0.2	2.4 ± 0.1
5%	L1	96.4 ± 12.6	ND	4.4 ± 0.3	4.9 ± 0.8	131.5 ± 12.5	112 ± 11.0	16.4 ± 1.6	0.2 ± 0.0	5.7 ± 0.3	0.68 ± 0.1	1.5 ± 0.1
	L2	99.4 ± 4.6	ND	4.2 ± 0.0	4.6 ± 0.0	125 ± 1.0	105.5 ± 1.5	15.7 ± 0.0	0.3 ± 0.0	5.3 ± 0.1	0.62 ± 0.0	1.5 ± 0.1
	L3	112 ± 0.0	ND	4.4 ± 0.0	4.9 ± 0.0	134 ± 0.0	112 ± 0.0	17 ± 0.0	ND	5.3 ± 0.0	0.6 ± 0.0	1.3 ± 0.0
	L4	102.5 ± 0.5	1.4 ± 0.0	4.4 ± 0.1	4.8 ± 0.0	125 ± 1.0	105 ± 1.0	16.5 ± 0.2	0.2 ± 0.0	5.3 ± 0.0	0.67 ± 0.0	1.6 ± 0.0
	L5	99.5 ± 4.5	5.3 ± 0.9	6.2 ± 0.2	6.5 ± 0.0	128.5 ± 0.5	110.5 ± 0.5	32.7 ± 1.1	ND	7.3 ± 0.3	0.66 ± 0.1	1.8 ± 0.1
2.5%	L1	66.1 ± 15.2	ND	2.7 ± 0.3	2.7 ± 0.4	84.5 ± 15.5	67.9 ± 12.3	8.5 ± 0.3	0.2 ± 0.0	3.5 ± 0.1	0.57 ± 0.1	1.3 ± 0.0
	L2	53.2 ± 15.4	ND	3.1 ± 0.4	2.5 ± 0.4	69.3 ± 2.7	56 ± 2.1	8.9 ± 1.4	ND	3.5 ± 0.5	0.57 ± 0.1	1.5 ± 0.2
	L3	64.2 ± 5.1	ND	2.6 ± 0.2	2.5 ± 0.0	73.2 ± 0.7	55.8 ± 0.3	7.7 ± 0.0	ND	3.5 ± 0.1	0.58 ± 0.0	1.1 ± 0.1
	L4	54.2 ± 2.5	ND	2.8 ± 0.1	2.4 ± 0.1	71.6 ± 0.4	56.6 ± 0.6	8 ± 0.1.0	ND	3.6 ± 0.1	0.69 ± 0.1	1.2 ± 0.1
	L5	55.6 ± 5.8	4.4 ± 1.5	4.3 ± 0.7	3.9 ± 0.7	72.3 ± 2.7	59.4 ± 2.0	15.9 ± 0.8	0.2 ± 0.0	5.2 ± 0.5	0.58 ± 0.0	1.5 ± 0.1

Table 2
Composition of raw NRD (initial composition) and permeates following membrane filtration: micro-filtration, ultra-filtration and nano-filtration. The highest concentration of each element is highlighted in bold.

	Raw NRD (Initial composition)	Micro-filtered NRD permeate	Ultra-filtered NRD permeate	Nano-filtered NRD permeate
pH	8.0	–	–	–
DW	5.94%	–	–	–
NH ₄ -N mg.L ⁻¹	4016	3146	1433	1940
P mg.kg ⁻¹	665	50.7	25.8	2.7
Ca mg.kg ⁻¹	6756	37 ± 0.6	33.7 ± 1.5	19.6 ± 0.3
K mg.kg ⁻¹	2015	1203 ± 12.5	815.2 ± 16.6	849.6 ± 22.7
Mg mg.kg ⁻¹	113.7 ± 7	6.0 ± 0.5	3.6 ± 0.5	ND
Na mg.kg ⁻¹	1150 ± 104.4	2146 ± 77.6	1300 ± 40.8	2230 ± 98.9
Al mg.kg ⁻¹	109.3 ± 2.1	20.9 ± 0.8	13.7 ± 0.9	20.9 ± 0.8
Cu mg.kg ⁻¹	1.6	0.6 ± 0.0	0.78 ± 0.1	2.3 ± 0.1
Fe mg.kg ⁻¹	6.2	8.2 ± 0.2	5.2 ± 0.21	2.3 ± 0.1
Zn mg.kg ⁻¹	32.9	1.1 ± 0.1	1.2 ± 0.2	1.3 ± 0.2

was deposited on ultra-carry discs and left to dry for 12 h. Samples were then processed for elemental analysis by XRF.

2.3. Membrane filtration – Complete processing of digestate

2.3.1. Experimental design and step-filtration process

Micro-filtration, ultra-filtration and nano-filtration were used to process the unsettled layer of raw NRD. Between each filtration step, the N, P and heavy metal composition of the NRD permeates were assessed.

Micro-filtration of NRD was implemented using a ceramic membrane at a pore size of 0.2 µm and a trans-membrane pressure ranging from 1.5 to 2.5 bars was applied (Koch membrane systems Inc.). The entire set-up comprised a 60L capacity tank connected to a pump drawing the NRD into the membrane for filtration. The permeate was collected at one end of the membrane and the remaining sludge (i.e. retentate) was pumped back into the tank and mixed with the remaining digestate for further filtration. Micro-filtration of NRD continued until the volume of retentate left in the tank reached 10 L. The obtained retentate was a thick and concentrated sludge.

The micro-filtered NRD permeate was then ultra-filtered at a pore size of 10 kDa (membrane: hollow fibre cartridge, GE Healthcare, UFP-10-E-6A). The ultra-filtration system functioned similarly to the one described for the micro-filtration step. A smaller retentate tank was used (20L) and ultra-filtration was stopped when the level of retentate collected reached 5L.

Micro-filtered NRD was also filtered using a nano-filtration membrane at a pore size of 500 Da. Nano-filtration was performed using frontal filtration in a high-pressure bench scale unit (HP4750, Sterlitech, Kent, WA, USA, NF270). The unit was continuously pressurised at 30 bar using nitrogen gas and a stirring speed of 300 rpm. The system had a maximum operation volume of 200 mL. A new membrane was installed between every trial to eliminate the risk of fouling and to avoid compromising permeate composition.

2.3.2. Nutrient and elemental composition analysis

The NH₄-N, P and elemental composition of permeates resulting from the different filtration steps detailed above were analysed as described in Section 2.2.3.

2.4. *Chlorella vulgaris* cultivation - biological processing of digestate

2.4.1. Processing of nano-filtered digestate at laboratory scale

The permeate resulting from the nano-filtration of NRD was used at different concentrations for the cultivation of *C. vulgaris*. Concentrations of 2.5% ([N] = 62.45 mg.L⁻¹; [P] = 0.07 mg.L⁻¹), 5% ([N] = 124.9 mg.L⁻¹; [P] = 0.13 mg.L⁻¹),

10% ([N] = 249.8 mg.L⁻¹; [P] = 0.27 mg.L⁻¹), 15% ([N] = 374.7 mg.L⁻¹; [P] = 0.39 mg.L⁻¹) and 20% ([N] = 499.6 mg.L⁻¹; [P] = 0.53 mg.L⁻¹) of nano-filtered NRD mixed with deionised water were tested. These conditions were compared against a 2.5% concentration ([N] = 101.3 mg.L⁻¹; [P] = 1.27 mg.L⁻¹) of micro-filtered NRD selected as a control.

Cultures of *C. vulgaris* were grown in triplicates for each digestate concentration, and the control, using 250 mL flasks, with a cultivation volume of 150 mL. An inoculum of 15 mL (10% of the total cultivation volume, corresponding to an OD 750 nm of 0.13) provided by the Centre for Sustainable Aquatic Research (Swansea University, UK) was used. Culture turbulence was provided by stirring and cultures were grown at a temperature of 25 °C under an illumination of 100 µmol.m⁻².s⁻¹ and a 12 Light:12 Dark photoperiod. Nano-filtered digestate was added at the beginning of the experiment, in the concentrations mentioned above, and cultivation occurred for eight continuous days without the addition of any other nutrient.

2.4.2. Processing of micro-filtered digestate at pilot-scale

The unsettled layer of undiluted digestate was chosen in this pilot-scale trial due to the simplicity of processing (settlement took place in a 60L tank for 24 h). Following settlement, micro-filtration was used to treat the unsettled layer of digestate prior to microalgal cultivation. Micro-filtration was selected due to practicality of the system, allowing for the processing of a large volume of NRD, necessary to this pilot-scale application. The micro-filtered permeate of digestate was diluted to 2.5% of the total photobioreactor volume (800L). This dilution corresponded to N and P concentrations of 101.3 mg.L⁻¹ and 1.27 mg.L⁻¹, respectively.

An inoculum of 80 L (10% of the PBR total volume, corresponding to an OD 750 nm of 0.13 and a DW of 0.168 g.L⁻¹) of *C. vulgaris* was used to start cultivation into a horizontal tubular photobioreactor, located in a greenhouse at Swansea University. The greenhouse temperature was maintained at 25 °C, natural light and photoperiod were used with an average light intensity throughout the experiment of 848.42 ± 64.92 µmol.m⁻².s⁻¹. The pH was maintained at 7.5 using automated CO₂ injection. *C. vulgaris* was cultivated for 51 days in semi-continuous mode. Harvesting occurred every 6 to 7 days to enable maximum N and P uptake from NRD while *C. vulgaris* was still in a state of exponential growth. Approximately 30% of the PBR total volume was harvested, followed by water renewal and digestate addition, maintaining a 2.5% concentration of micro-filtered NRD in the cultivation system.

2.4.3. Growth measurements

2.4.3.1. Optical density at 750 nm. Growth rate of *C. vulgaris* was assessed through daily measurements of the absorbance at 750 nm for the laboratory scale experiment and every other day

for the pilot-scale trial. Absorbance at 750 nm was utilised to measure biomass as this specific wavelength avoids light absorption by pigments, and so can be treated as a light scattering measurement (Chioccioli et al., 2014). Growth rate was calculated during the exponential phase of growth using the following equation:

$$\mu = \ln(OD_2/OD_1)/(t_2 - t_1) \quad (2)$$

Where μ is the specific growth rate (day^{-1}), OD_1 and OD_2 are the optical density measured at 750 nm at time 1 (t_1) and time 2 (t_2).

2.4.3.2. Dry weight. Dry weight (in g.L^{-1}) of produced biomass was assessed in the pilot-scale trial, where the volume of culture was sufficient for regular sampling of *C. vulgaris*. Three replicates of 20 mL each were sampled every other day and dry weight was assessed as described in Section 2.2.2.

2.4.3.3. Nitrogen and phosphorus content. Nitrogen and phosphorus were measured in the culture supernatant every other day in the pilot-scale trial and assessed according to the method detailed in Section 2.2.3.

2.5. Statistical analysis

Statistical analysis was carried out on settlement rate, OD750 nm, dry weight, particle count, $\text{NH}_4\text{-N}$ and P concentrations, and elemental analysis using the R project software. The OD 750 nm and dry weight were also analysed for the microalgae cultivation trials. Crossed factors ANOVAS were carried out on normally distributed data and normality was determined using Shapiro tests. When statistical significance was found, post hoc Tukey tests were implemented. Results were deemed significant when p-value was below 0.05.

3. Results and discussion

3.1. Dilution and settlement – Partial processing of digestate

3.1.1. Settlement rate of diluted NRD

As shown in Fig. 1, a faster settlement rate was observed in highly diluted digestate in comparison to lower levels of dilution tested. Specifically, digestate settled at an average rate of $0.314 (\pm 0.005) \text{ cm.h}^{-1}$ at a 2.5% concentration while the settlement was only at a rate of $0.04 (\pm 0.002)$ and $0.057 (\pm 0.003) \text{ cm.h}^{-1}$, respectively for a 20% concentration and 100% concentration (*i.e.* control). Both the 20% concentration of digestate and the control presented

a low and constant settlement rate during the 24 h of the experiment (Fig. 1) and showed very little sedimentation of the NRD beyond four hours. On the other hand, for a 2.5% concentration of digestate, a high settlement rate of $1.553 (\pm 0.05) \text{ cm.h}^{-1}$ was recorded at the beginning of the experiment, only to reach a plateau after four hours (significantly different from the control, p-value < 0.05); similar results were found for a 5% concentration (Fig. 1). These results demonstrated that dilution significantly accelerated the settlement rate of NRD, increasing the potential for separation of solid and liquid fractions. A direct correlation can be made between these results and a reduced heavy particles content in the diluted digestate, responsible for an enhancement of settlement properties. Furthermore, results indicated that sedimentation occurred during the first few hours of settlement, showing that settlement techniques would represent an efficient and low-cost process for higher amounts of digestate within a shorter processing time.

3.1.2. Dry weight, turbidity and particle distribution of settled layers

Dry weight, turbidity and particle distribution were significantly higher in L5 for all tested conditions (p-value < 0.01, Tukey test: $L1 = L2 = L3 = L4 < L5$). Similarly, DW was three-fold higher in L5 (12.18 g.L^{-1} ; Fig. 2) compared to the other four layers (ranging from 3.66 to 4.01 g.L^{-1} ; Fig. 2). Similar results were observed for optical density and particle count, where OD is an indicator of turbidity and particle count and DW are a direct assessment of the particulate matter present in a sample. Therefore, these results showed that the vast majority of particles present in the column were found in L5 at the end of the experiment, confirming that settlement and decantation occurred at a significant degree during the time of experiment. Findings from de Godos et al. (2009) reported that when a swine manure digestate had been settled at a residence time of five days, the total of suspended solids was reduced by 70% in the digestate column. In the present study, sedimentation of the Langage AD digestate (originating from the anaerobic digestion of kitchen food waste) was faster, exhibiting that sedimentation time can depend on the nature of the settled digestate.

3.1.3. Nutrient analysis of settled layers

Results of nutrient analysis showed a maximum $\text{NH}_4\text{-N}$ concentration of 6928 mg.L^{-1} in L4, in the control (*i.e.* undiluted digestate, Fig. 3a). P content was similar in all collected layers for digestate concentrations of 2.5%, 5% and 10% (p-value < 0.05). Recalculated values of $\text{NH}_4\text{-N}$ and P content from treated digestate did not show

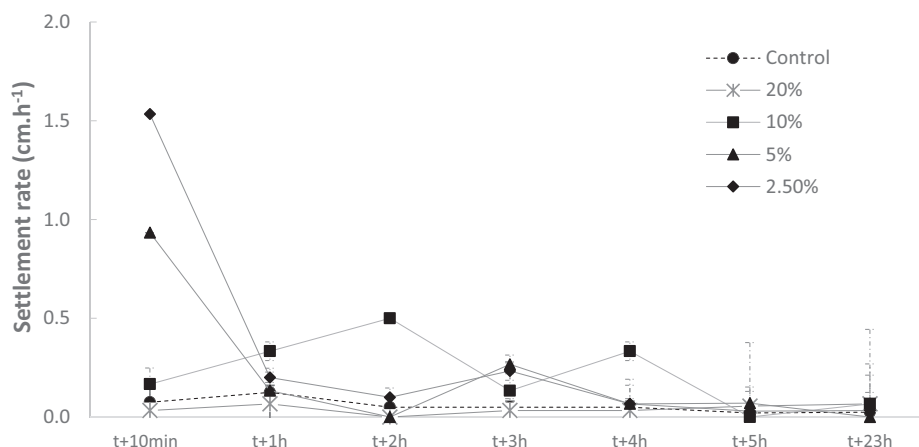


Fig. 1. Settlement rate of diluted digestate at concentrations of 2.5%, 5%, 10% and 20% compared to a raw undiluted digestate (control). Settlement rate measured from $t + 10 \text{ min}$ to $t + 23 \text{ h}$. Error bars represent standard deviation of data on three replicates for diluted digestate and four replicates for the control.

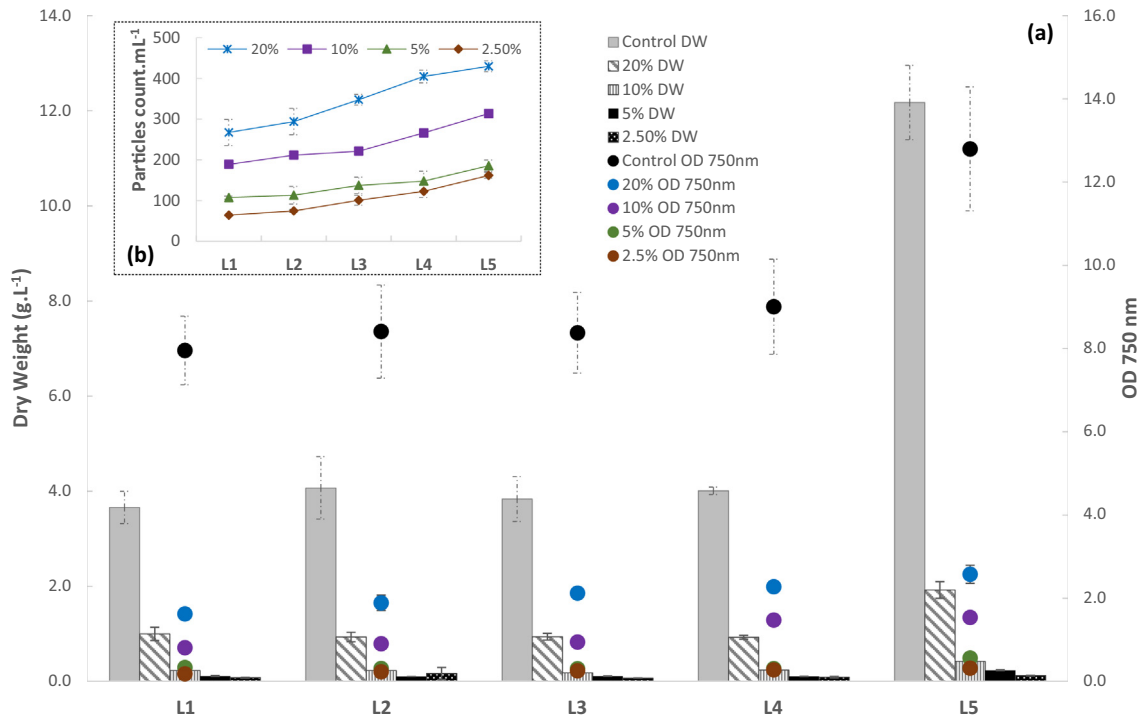


Fig. 2. (a) Dry weight (left axis, bar plot) and OD 750 nm (right axis, coloured dots) and (b) particle count data of diluted digestate at concentrations of 2.5%, 5%, 10% and 20% for 5 layers collected after 24 h of experiment. Dry weight and OD 750 are compared to a raw undiluted digestate (control). Error bars represent standard deviation of data on three replicates for diluted digestate and four replicates for the control.

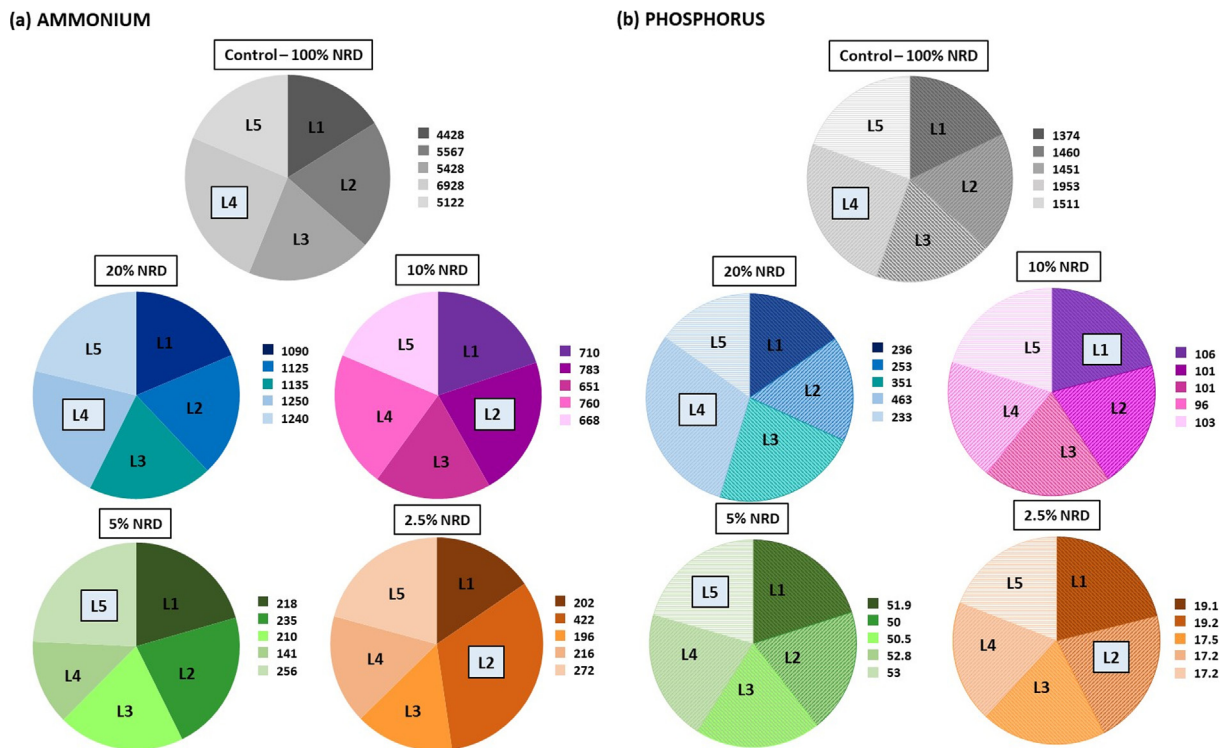


Fig. 3. Ammonium (a) and Phosphorus (b) (in mg.L⁻¹) content of diluted digestate at concentrations of 2.5%, 5%, 10% and 20% compared to a raw undiluted digestate for five layers collected after 24 h of experiment. Layers presenting the higher concentration of nitrogen or phosphorus are outlined in a blue frame. Standard deviations were not significant and are not shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

an increase of either nutrient, except for the NH₄-N content for a 2.5% concentration, where the highest concentration of NH₄-N measured in L2 was 422 mg.L⁻¹. This result corresponds to an

equivalent concentration of 16.88 g.L⁻¹ for a 100% concentration of digestate, which is 2.4 times higher than the highest measured concentration in the control. This suggests that a high level of

water dilution could have caused a release of $\text{NH}_4\text{-N}$. However, it is unlikely that dilution alone was responsible for such an increase in the $\text{NH}_4\text{-N}$ content, and it could be argued that digestate was still active to some extent, resulting in protein degradation, producing $\text{NH}_4\text{-N}$ ions measured in the study (Jokela and Rintala, 2003). The increase in P was consistent with findings from the literature, where water dilution has been shown to dissolve some of the solid particles binding P, and releasing the compound in the liquid fraction of digestate (Gerardo et al., 2015). Furthermore, digestate dilution was found to solubilise mineral precipitates and release other compounds of interest (Wahal and Viamajala, 2016). Dilution has been widely used as a digestate treatment, especially when NRD was used as a waste based medium for microalgal cultivation (Abu Hajar et al., 2017; Franchino et al., 2016; Lu et al., 2015). In these studies, dilution was a means to reduce the load of some potentially toxic elements, such as $\text{NH}_4\text{-N}$; this work demonstrated that dilution could also release important nutrients for microalgal cultivation and allowed for some manipulation of these nutrient concentrations.

3.1.4. Elemental analysis of settled layers

Results from the elemental analysis on each of the collected layers of diluted and undiluted NRD at the end of the 24 h experiment

are compiled in Table 1. Each element concentration was significantly higher in L5 (p-value < 0.01, Tukey test: L1 = L2 = L3 = L4 < L5) compared to the remaining layers (L1 to L4) that were not significantly different from each other. For example, in the control, calcium and iron were both significantly higher (p-value < 0.01) with respective concentrations of 590.7 mg.kg^{-1} and 128.7 mg.kg^{-1} in L5, while the concentrations in the remaining layers ranged from 176.3 to 204.3 mg.kg^{-1} and 68.6 to 80 mg.kg^{-1} for calcium and iron, respectively (Table 1). Similar results were found across the range of tested dilutions.

Results of elemental analysis demonstrated that dilution contributed to the release of some elements from the solid fraction to the liquid fraction. To illustrate, zinc had a concentration of 1.5 mg.kg^{-1} in L5 for a digestate diluted down to 2.5%, corresponding to a recalculated concentration of 60 mg.kg^{-1} in a 100% NRD. This is 5 times higher than the amount of zinc measured in L5 for the control ($\text{Zn} = 12.9 \text{ mg.kg}^{-1}$, Table 1). The same observation was made for sodium, aluminium, sulphur, chlorine, potassium, calcium, iron and copper. However, some elements, such as magnesium and manganese, were not detected in NRD diluted down to 2.5%, 5% and 10% (Table 1). Heavy metals, especially zinc and copper, are toxic to photosynthetic organisms at high concentration (Papadimitriou et al., 2008) but are essential oligo-elements

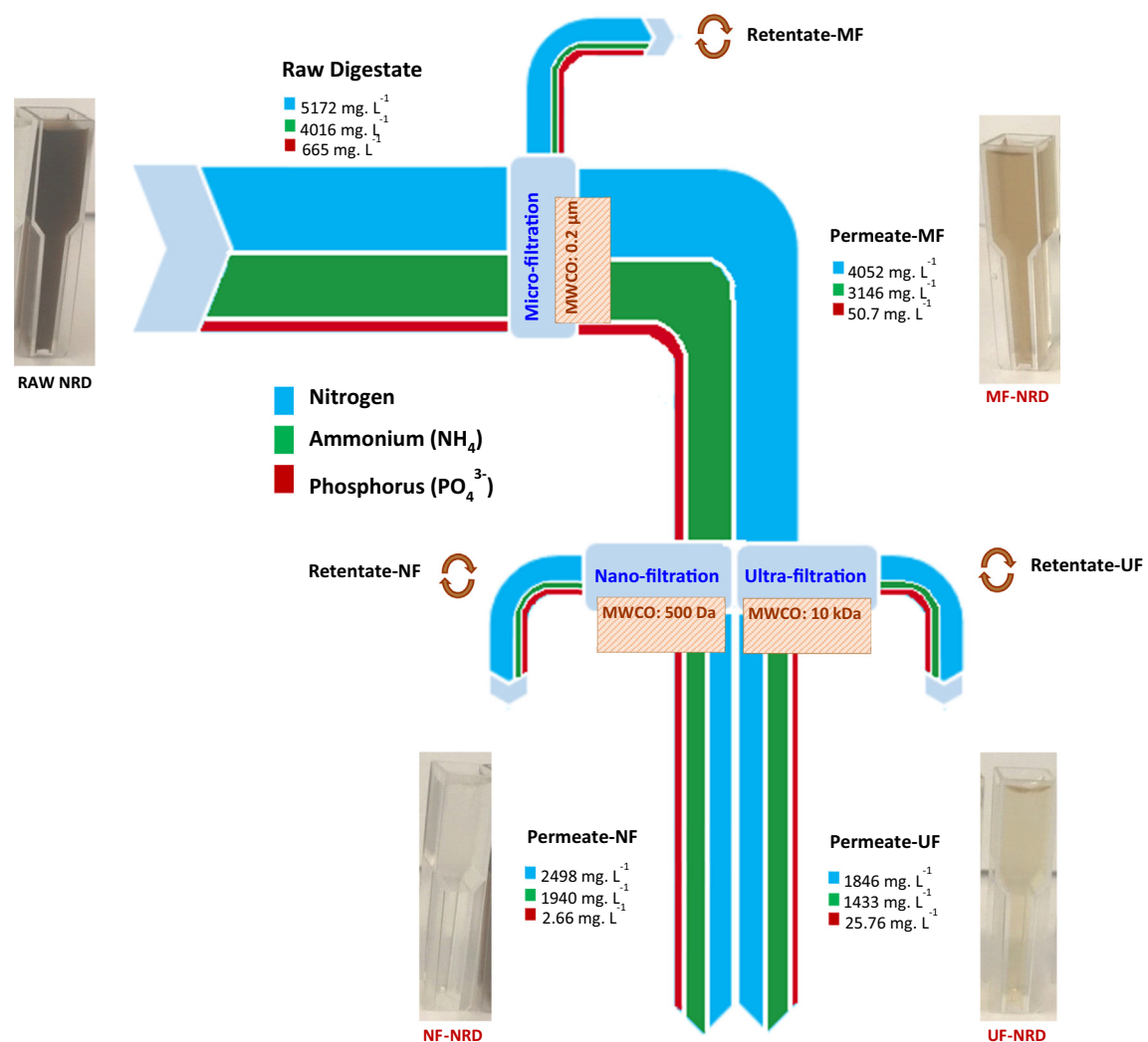


Fig. 4. Concentration flow diagram of the membrane filtration process for nutrient-rich digestate for total nitrogen (blue), ammonium (green) and phosphorus (red). Arrow thickness represents relative concentrations between the three nutrients. MF: micro-filtration; UF: ultra-filtration; NF: nano-filtration. Permeate composition is shown after every filtration step, circular arrows indicate the recirculation of retentate during the filtration process. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

when supplemented at the right concentration, including in microalgal cultivation systems (Kropat et al., 2015).

Analytical results indicate that the combination of dilution and settlement as a partial processing of digestate was a promising technique to separate liquid and solid fractions of digestate. Additionally, NRD sedimentation occurred in a few hours, resulting in a settled layer of NRD (or solid fraction), transportable at a lower cost once dried, and with the potential to be used in fertiliser formulation (Alburquerque et al., 2012). In the unsettled layer of digestate, results indicated that macronutrients (N and P) and micronutrients (zinc, calcium, iron) were all released, and these compounds are all essential to microalgal growth. Hence, there is potential to use this partial processing of NRD as part of the upstream process to produce a waste-based medium for microalgae cultivation. However, it is important to highlight that only high levels of dilutions proved to be beneficial for this purpose, inducing consequent water usage when scaled-up for mass cultivation of microalgae. If dilution is to be considered in large-scale cultivation systems, water from culture dewatering (i.e. harvesting, downstream process) could be recycled and used for the dilution of digestate for several utilisation cycles, reducing the cost linked to water usage and avoiding water penalties. Additionally, despite evidencing benefits for microalgal growth in terms of nutrient release, a significant number of particulates were remaining in the unsettled layers of diluted and raw digestates, particulates that provide a substrate for the development of contamination, potentially harmful to microalgal growth (Xia and Murphy, 2016). To illustrate, when testing the growth of *Chlorella sp.* on raw digestate from a municipal waste treatment facility, Cho et al. (2011) found that bacterial load and suspended solids were responsible for a lack of growth in the microalgae. Light penetrability was also very limited in a raw digestate, compromising photosynthetic performances by microalgal cultures (Marazzi et al., 2017; Rusten and Sahu, 2011). Here, while dilution improved transparency of the digestate and reduced the particulates load, these were nevertheless sub-optimal for microalgal growth, bringing a risk to compromise cultures, especially when considering large-scale systems and commercial viability of biomass production. Hence, there is an argument for exploring additional treatments of digestates following settlement and dilution, in an effort to maximise microalgal production. Filtration of the unsettled layer of digestate can be explored to remove remaining particulates and associated contaminations and increase light penetrability for optimal microalgal growth.

3.2. Membrane filtration – Complete processing of digestate

The N, $\text{NH}_4\text{-N}$ and P content of NRD following each filtration step (micro-filtration, ultra-filtration and nano-filtration) is presented in Fig. 4. Elemental analysis is summarised in Table 2. Fig. 4 shows that most of the P was retained at the micro-filtration step, with a concentration of only 50.7 mg.L^{-1} measured in the permeate. Concentrations of 25.76 mg.L^{-1} and 2.66 mg.L^{-1} were found in the ultra-filtration and nano-filtration permeates respectively, showing a significant decrease in comparison to the raw digestate, which had a P concentration of 665 mg.L^{-1} . Results demonstrated that most of the P was bound to the solid fraction of NRD, as most of the solid particles were removed during micro-filtration. Findings from Tambone et al. (2017) confirm this result, as they showed that dry matter and nutrients were concentrated in the solid fraction of processed digestate. A concentration of 4052 mg.L^{-1} of N was measured in the permeate after micro-filtration and concentrations of 1846 mg.L^{-1} and 2496 mg.L^{-1} were found in the ultra-filtration and nano-filtration permeates, respectively. Thus, some of the N was lost during the different filtration steps, but concentration stayed relatively high, even following

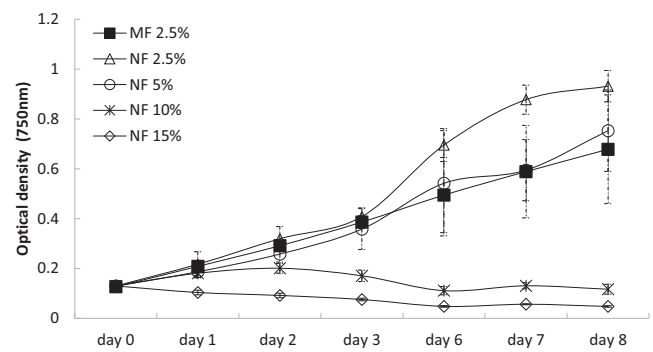


Fig. 5. OD 750 nm measurements in *C. vulgaris* cultures grown on different concentrations of nano-filtered digestate and a micro-filtered digestate control. Error bars represent standard deviation of data on three replicates.

nano-filtration. The obtained results were consistent with results from Adam et al. (2018), who recovered 53% of $\text{NH}_4\text{-N}$ in nano-filtered digestate. The significant reduction in P after each filtration step, resulted in a N to P ratio of 80, 71 and 939 in the micro-filtered, ultra-filtered and nano-filtered permeates, respectively.

In this study, while filtration was very efficient in cleaning the NRD by removing particles and maintaining suitable levels of N for microalgal cultivation, this method significantly reduced the amount of bioavailable P. However, studies showed that using membrane filtration in association with chemical pH adjustment could increase the amount of P released in the permeate. Gerardo et al. (2015) demonstrated that P could be recovered from dairy manure digestate through a series of diafiltration (i.e. addition of equal amounts of water for effective dialysis of solutes) and that pHs of 3 and 7 led to recovery of 96.4% and 97.2% of P respectively. Hence, there are methods available to increase the amount of P recovered after NRD treatment, and despite incurring cost, these methods can be considered to improve the N to P ratio and tailor it to microalgal needs.

While P appeared to decrease consistently with each filtration step, other compounds were found in higher concentrations in the nano-filtered permeate, in comparison to permeates resulting from ultra-filtration and micro-filtration. As an example, N was 1.35 times higher in the nano-filtered product compared to the ultra-filtration permeate. Elemental analysis (Table 2) also showed that sodium, copper and zinc were found in higher concentrations in the nano-filtered permeate when compared to both ultra-filtered and micro-filtered permeates. The nano-filtration process has the characteristic to concentrate metals due to the small pore size of the membrane, and the divalent charge of the species interacting with the charged active layer on the membrane surface. This specificity could explain why the aforementioned elements were found in higher concentrations following nano-filtration (Al-rashdi et al., 2013; Gherasim and Mikulá, 2014).

Analysis revealed that nano-filtration was the only process resulting in a colourless permeate (Fig. 4), and magnesium was the only compound not detected in the nano-filtered digestate in comparison to micro and ultra-filtered NRDs. There is no evidence suggesting that magnesium is solely responsible for digestate colouration. Hence, it is likely that the digestate colour resulted from the interaction of several compounds, such as humic substances, which are large organic compounds and were not assessed in this research. Furthermore, it is challenging to assess the exact compound (or mixture of), responsible for digestate colouring, as digestate colour can also vary between AD facilities due to the nature of the waste input (Marcilhac et al., 2014).

Membrane filtration was an efficient treatment of digestate and allowed for its complete processing by producing a clear liquor

after nano-filtration, potentially dischargeable to receiving waters (Fuchs and Drosig, 2013). Additionally, the nutrient segregation following each filtration step enables the tailoring of nutrients for specific fertiliser utilisation, contributing to the valorisation of raw digestate. However, this multi-step technology is costly and not readily scalable when considering the current need for processing vast amounts of excess NRD produced across North West Europe. Using treated NRD as a medium for microalgae cultivation has potential to provide a solution to alleviate NRD processing cost and add value to excess NRD, currently underused and under valorised by the AD sector.

3.3. *Chlorella vulgaris* growth trial

3.3.1. Results of laboratory scale cultivation

Nano-filtered digestate was used as nano-filtration was the only process successful at removing the NRD colour, allowing for the testing of a greater range of digestate concentrations. The OD at 750 nm as a measurement of *C. vulgaris* growth showed significant differences between some of the concentrations tested (p -value < 0.01, Tukey test = NF 2.5 > NF5 = MF2.5 > NF10 > NF15). *C. vulgaris* grew best on a 2.5% concentration of nano-filtered NRD with a maximum OD of 0.93 reached after 8 days of experiment and a growth rate of 0.46 day^{-1} (Fig. 5). A 5% concentration of nano-filtered NRD showed the second-best results with a final OD of 0.75 (growth rate of 0.19 day^{-1}). Similar performances were observed for the micro-filtered NRD control (growth rate of 0.17 day^{-1}). Neither 10% nor 15% concentrations of nano-filtered digestate yielded significant growth of *C. vulgaris* (Fig. 5).

The N:P ratio in both micro and nano-filtered digestates was different to the recommended N:P ratio for efficient growth (Redfield ratio of 16:1-N:P; Geider and La Roche, 2002; Rhee and Gotham, 1980). The significant reduction of P resulting from the membrane filtration process was responsible for the N:P ratio val-

ues. Some studies reported supplementing treated digestate with artificial medium to improve microalgal growth. For example, Hollinshead et al. (2014) supplemented digestate from municipal waste sludge with BG11 medium (at a ratio of 1:4-digestate:BG11) and obtained a growth rate of 0.14 day^{-1} in *Synechocystis* sp. Additionally, unpublished data from ALG-AD project partners reported that an addition of monosodium phosphate (NaH_2PO_4) to digestate sourced from pig manure (initial P concentration of 25 mg.kg^{-1}) resulted in a cell count of $1.5 \times 10^8 \text{ cells.mL}^{-1}$ in cultures of *Auriantiochitrium mangrovei*. However, P supplementation results in additional production costs, especially at higher cultivation scales.

Magnesium was not detected in the nano-filtered NRD, however, magnesium is required for microalgal growth (Becker, 1994), as it acts as a chelator in the chlorophyll complex, which was found to be a limiting factor for microalgae development (Park et al., 2010). Another study supported these findings and identified magnesium as a limiting factor for the growth of microalgae on digestate sourced from pig slurry (Bjornsson et al., 2013).

In this work, low concentrations of NRD were found to be more suitable for the cultivation of *C. vulgaris*, however more work is needed to tailor specific nutrients and oligo-elements necessary to facilitate microalgal growth. Indeed, results indicated that micro and macro nutrients such as P and magnesium could be supplemented to improve growth further, but this work confirmed that *C. vulgaris* could grow on processed digestate and evidenced potential for larger scale applications and the consequent valorisation of excess NRD into microalgal biomass. Furthermore, while comparing nano-filtered digestate which was colourless (Fig. 4) and micro-filtered digestate that had retained some colour (Fig. 4), results showed that growth was similar for a 2.5% diluted micro-filtered digestate and a 5% diluted nano-filtered digestate. Based on this result, it could be assumed that while using a highly-

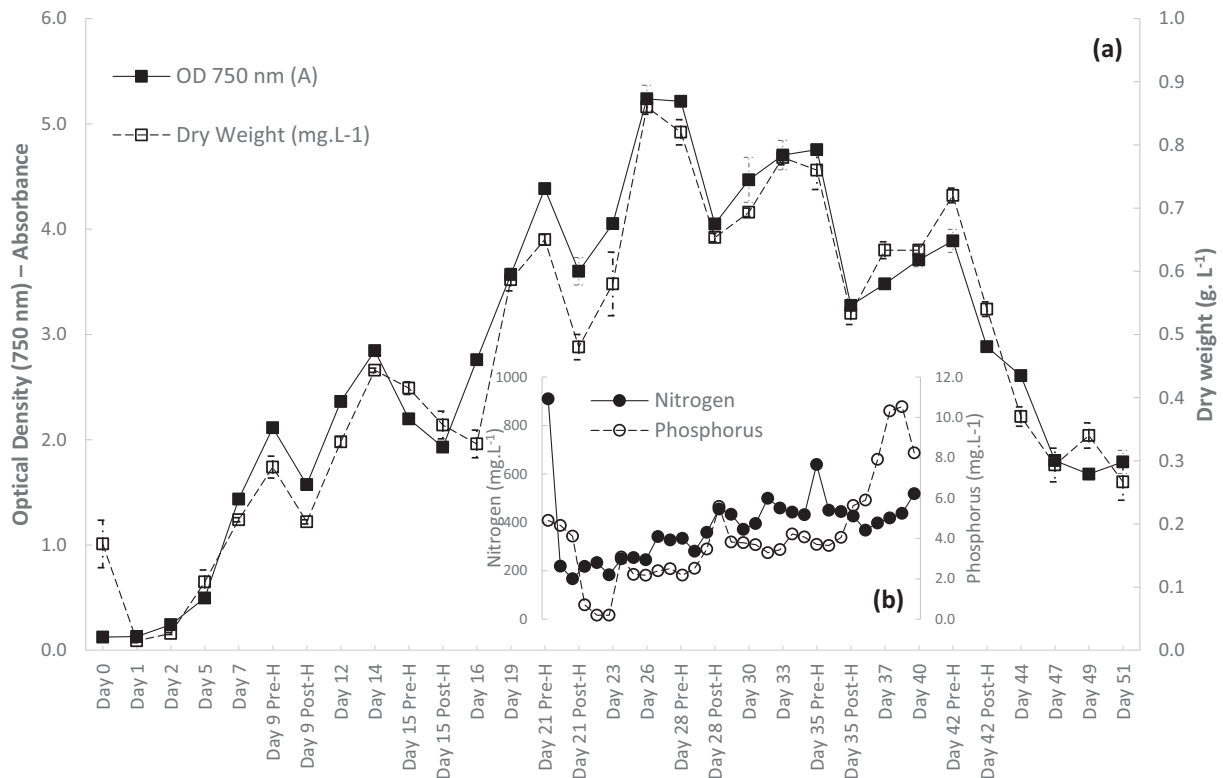


Fig. 6. (a) OD 750 nm measurements (left axis) and dry weight measurements (in g.L^{-1} , right axis) in *C. vulgaris* grown in a 800 L photobioreactor. (b) Nitrogen (left axis) and phosphorus (right axis) in mg.L^{-1} measured in the culture supernatant. Pre-H: pre-harvest; Post-H: post-harvest. Error bars represent standard deviation of data on three replicates.

diluted and filtered digestate, colour was not impacting *C. vulgaris* growth negatively.

Some studies however, demonstrated that digestate colour could have a detrimental impact on microalgal growth and associated removal of N due to limitation of light availability for photosynthesis (Marcilhac et al., 2014). Hence, a high level of dilution (implemented to reduce the load of $\text{NH}_4\text{-N}$ in the NRD), can also be beneficial to microalgal growth by attenuating NRD colour and avoiding compromising photosynthetic performances.

3.3.2. Results of pilot-scale cultivation

C. vulgaris was cultivated for 51 days in an 800 L photobioreactor in semi-continuous mode under a concentration of 2.5% micro-filtered digestate. Harvesting and water renewal occurred every 6 to 7 days. Temperature, pH, and light intensity were stable throughout the time of cultivation. Results of OD at 750 nm and dry weight showed that growth was continuous for 28 days with an average growth rate of 0.143 day^{-1} (a maximum growth rate was recorded at day 3: 0.620 day^{-1} , and maximum OD 750 nm: 5.24). Productivity averaged at $47.57 \text{ mg.L}^{-1}.\text{day}^{-1}$ (with a maximum productivity recorded at day 17: $93.33 \text{ mg.L}^{-1}.\text{day}^{-1}$, and maximum dry weight: 0.86 g.L^{-1}) (Fig. 6a). Decrease in growth was observed after 28 days of cultivation, reaching a final OD 750 nm of 1.79 and a final dry weight of 0.267 g.L^{-1} . N and P concentrations dropped significantly during the two first days of cultivation and a slight drop in both concentrations was observed following each addition of microfiltered digestate, showing that both N and P were used by *C. vulgaris* (Fig. 6b). However, data from the full duration of the experiment revealed a slow increase in both N and P, showing that consumption of nutrients from the micro-filtered NRD by *C. vulgaris* was only partial, resulting in an accumulation of both compounds in the cultivation system.

The decline of *C. vulgaris* in culture was concomitant with the increase in N and P in the cultivation medium. $\text{NH}_4\text{-N}$ has been found to be toxic to microalgae in high concentrations (maximum tolerance of total ammoniacal nitrogen of 500 mg.L^{-1} reported for *C. vulgaris* (Uggetti et al., 2014; Xia and Murphy, 2016)). N had a final concentration of 518.4 mg.L^{-1} (Fig. 6b), corresponding to a $\text{NH}_4\text{-N}$ concentration of 402.5 mg.L^{-1} . The high concentration and accumulation of $\text{NH}_4\text{-N}$ in the system could be an element of response for the decline of *C. vulgaris* after 28 days of cultivation. Adjusting the addition of NRD in the culture could counteract the $\text{NH}_4\text{-N}$ accumulation, for example in Marazzi et al. (2017), authors were only adding digestate (from piggery manure) when $\text{NH}_4\text{-N}$ was fully depleted in the cultivation system, and the concentration of N was maintained below a maximum of 160 mg.L^{-1} , thus avoiding toxicity linked to an accumulation of $\text{NH}_4\text{-N}$.

Heavy metals such as aluminium, copper and zinc are present in significant amounts in digestate, and microalgae have been widely studied for their capacity to absorb heavy metals, contributing to their popularity in bioremediation technologies (Kropat et al., 2015; Papadimitriou et al., 2008). However, in the case of digestate, microalgae must cope with a mixture of different metals, and other organic compounds such as humic substances. Consequently, toxicity could have been induced by the accumulation and interference between such compounds that are absorbed by microalgae and potentially interfere with growth and cell development (Al-rub et al., 2006; Mehta and Gaur, 2008; Zhou et al., 2012).

In addition, bacterial contaminations are likely to occur in large-scale systems (Subashchandrabose et al., 2011) and could have compromised the growth of *C. vulgaris* by competing with the microalgae for nutrients, but also by producing algicidal compounds responsible for the lysis of microalgal cells (Mayali and Azam, 2004). While aiming to bioremediate digestate using microalgae, an artificial consortium of several species could be considered to improve yields and use contaminations as an advantage, as they are highly likely to occur

in large-scale systems. Further down the line, microalgae-bacteria consortia could be explored, as bacteria can contribute to $\text{NH}_4\text{-N}$ remineralisation and improve remediation performance by microalgae (Hu et al., 2018; Luo et al., 2017; Sniffen et al., 2016).

Despite a growth decline after 28 days of cultivation, *C. vulgaris* presented regular growth with a high dry weight, demonstrating that cultivation of *C. vulgaris* at a large scale using digestate as a feedstock is a promising technology with the potential to convert excess nutrients from digestate into valuable biomass. Knowing that the decline in growth was likely linked to an accumulation of $\text{NH}_4\text{-N}$, and heavy metals in the cultivation system, solutions such as a complete harvest of the biomass and renewal of the medium could be implemented. An adjustment of the digestate addition to maintain an $\text{NH}_4\text{-N}$ concentration below toxicity level, could also contribute to an improvement of growth beyond the 28 - days of cultivation achieved in this study.

4. Conclusions

In this work, it was evidenced that dilution combined with settlement provided a low-cost method for processing digestate, allowing for transportation of smaller volumes of NRD to areas outside of the NVZs legislation. Release of compounds for fertiliser usage was also induced by dilution, including valuable compounds for microalgal cultivation. Membrane filtration was efficient at separating liquid and solid fractions of NRD and at recovering nearly 95% of both N and P, while significantly reducing heavy metals in the NRD permeates. Nano-filtration produced a clear liquor potentially dischargeable into the environment, showing that nano-filtration could clean-up NRD. This process remains costly and is not readily scalable for industrial uses, reinforcing the need for NRD valorisation by finding new markets for excess nutrients. Cultivation of *C. vulgaris* at pilot scale (800L) on micro-filtered digestate was successful for 28 continuous days, demonstrating the potential for biological processing of NRD to enable the conversion of excess nutrients into valuable biomass. Additional research would benefit the studied processes, for example by incorporating a pre-membrane filtration dilution step for large-scale applications, improving further microalgal growth conditions and yields by enhancing nutrient balance. The studied technology is scalable and can lead to the production of high amounts of microalgal biomass. *C. vulgaris* is a GRAS specie (Generally Regarded as Safe) for the food and feed sector, hence the production of microalgae using excess nutrients from digestate has the potential to open new markets for the AD sector and add value to increasing volumes of produced digestate.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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