White and infrared light continuous photobioreactors for resource recovery from poultry processing wastewater – a comparison

Tim Hülsen[a*], Kent Hsieh[a], Stephan Tait[a], Edward M. Barry, Daniel Puyol[b], Damien J. Batstone[a]

[a]Advanced Water Management Centre, Gehrmann Building, The University of Queensland, Brisbane,
Queensland 4072, Australia

[b] Group of Chemical and Environmental Engineering, School of Experimental Sciences and Technology, King Juan Carlos University, Mostoles, Spain

*Corresponding Author:
Tim Hülsen
Advanced Water Management Centre
The University of Queensland
St Lucia, Brisbane, 4072, Australia
Phone: +61 (0)7 33467209
Fax: +61(0)733654726
E-mail: t.huelsen@awmc.uq.edu.au

Abstract

Concentrated wastewaters from agricultural industries represent a key opportunity for the upcycling of organics, nitrogen and phosphorus to higher value products such as microbial protein. Phototrophic or photosynthetic microbes very effectively capture input organics and nutrients as microbial protein. This study compares purple phototrophic bacteria (PPB) and microalgae (photosynthesis) for this purpose, treating real, high strength poultry processing wastewater in continuous photo bioreactors utilising infrared (IR) and white light (WL) respectively. Both reactors could effectively treat the wastewaters, and at similar loading rates (4 kgCOD $m^{-3}d^{-1}$). The infrared reactor (IRR) was irradiated at 18 W m^{-2} and the white light reactor (WLR) reactor at 1.5-2 times this. The IRR could remove up to 90 % total chemical oxygen demand (TCOD), 90 % total nitrogen (TN) and 45 % total phosphorus (TP) at 1.0 d hydraulic retention time (HRT) and recover around 190 kg of crude protein per tonne of influent COD at 7.0 kWh per dry $tonne^{-1}$ light input, with PPB dominating all samples. In comparison, the WLR removed up to 98% COD, 94 % TN and 44 % TP at 43 - 90 % higher irradiance compared to the PPB reactor. Microalgae did not dominate the WLR and the community was instead a mix of microbes (algae, bacteria, zooplankton and detritus – ALBAZOD) with a production of approximately 140 kg crude protein per tonne influent COD.

Keywords: agri-industrial, resource recovery, wastewater, photobioreactor, PPB

1.Introduction

With the shift in wastewater treatment from pollutant removal to resource recovery, concentrated wastewaters from agricultural industries are major “low hanging fruit” opportunities to recover resources (Angenent et al. 2004). This is because they represent a concentrated source of nutrients and organic carbon, with less variety of microbial, metal, and organic contaminants generally present in domestic wastewater. Use of industrial wastewater also avoids input of human specific pathogens commonly present in domestic wastewater, as well as other regulatory barriers governing input of domestic sewage into the human food chain. This mandates that on-site human sewage does not enter the industrial wastewater stream, but instead be directed to the urban sewer system. In this context and compared with traditional treatment, resource recovery from agri-industrial streams can a) achieve lower wastewater treatment costs by offsetting costs with value from generated products (e.g. fertilizer, proteins, biogas) (Corominas et al. 2013, Mo and Zhang 2013), b) lower product production costs of these products by using recycled raw materials in the wastewater (such as organics, nitrogen and phosphorus) (Matassa et al. 2015, Foley et al. 2010) and c) reduce environmental impacts by substitution of nitrogen (N) otherwise generated from the Haber Bosch process (Rockström et al. 2009). Economic analysis has demonstrated that resource recovery technologies can be economically competitive with traditional treatment processes, particularly where a higher value, high quantity product is generated (Burgess et al. 2015).

A key option for efficient resource recovery from wastewater is the partitioning of soluble organic matter and nutrients into a concentrated separable solid phase

(Batstone et al. 2015). This can be achieved via biological assimilation, where organic matter, N and phosphorus (P) are removed simultaneously, preferably non-destructively, and incorporated into protein-rich biomass. Both, microalgae and purple phototrophic bacteria (PPBs) are promising candidates, achieving biomass yields close to unity (Buitrón et al. 2018, Hülsen et al. 2016a) and high protein contents of up to 60% by dry weight (Shipman et al. 1975, Brown 1991).

Microalgae have been extensively studied for the production of feed and feed additives, e.g. for fish, pets and farm animals as well as humans (Spolaore et al. 2006) and are mainly produced in open high rate algal ponds (HRAP) developed almost 60 years ago (Oswald and Golueke 1960), although tanks, tubes and fermenters have been applied (Borowitzka 1999). To date, full-scale microalgae production, mainly to produce biodiesel is almost exclusively carried out in HRAP where closed photobioreactors remain too expensive for efficient mass production (Savage 2011, Park et al. 2011).

Compared to microalgae, PPBs have been far less studied which concerns the production as well as the application as feed. A few studies are dealing with PPB as feed additive for aquaculture (Chowdhury et al. 2016, Banerjee et al. 2000, Loo et al. 2015) and chicken (Ponsano et al. 2004) where axenic PPB cultures, grown on artificial media were utilized. In this context, the carotenoid and vitamin content in PPBs has been researched in more detail (Takeno et al. 1999) but larger scale production has only been reported in a handful of papers in mixed cultures with microalgae, also in open ponds (Kobayashi et al. 1971, Kobayashi and Tchan 1973). Reports of large-scale probiotics production in China (Qi et al. 2009), which contains fractions of PPB and is not selective and therefore not considered in the single cell protein context.

Energy input as light is a key consideration for both microalgae and PPB, with rather inefficient use of photons for oxygenic and anoxygenic photosynthesis (<10% (Melis 2009, Adessi and De Philippis 2014)). PPB also grow effectively on volatile fatty acids (VFAs) but can utilize a variety of other organic substrates such as sugars and alcohols for photoheterotrophic metabolism (Imam et al. 2013, Kim et al. 2004). Algae preferentially utilizes CO_2 (Azov et al. 1982, Craggs et al. 2011) to synthesize organics via photoautotrophic growth in an energy intensive process (Turpin 1991) compared with low-energy direct assimilation of wastewater derived organic matter. Agri-industrial wastewater generally comprises a vast variety of carbon substrates (e.g. 55.3% fat, 27.1% protein, 7.4% carbohydrates as a % of dry matter in poultry processing wastewater (Kiepper et al. 2008)), not necessarily preferred for growth, and in most cases requires fermentative or oxidative organisms to convert these complex organics to organic acids or CO_2 respectively. Reducing equivalents/organics (chemical oxygen demand (COD)) might not be removed, but instead transformed, first to organic acids (Zhang et al. 2009) and subsequently to microbial biomass by PPB. Competition for macronutrients can become secondary due to carbon limitations.

In both, microalgal and PPB systems, community stability is crucial for effective wastewater treatment and consistent product generation. A microbial community can be syntrophic, parasitic, competitive or a combination of these, with changes in

wastewater composition resulting in community shifts (Schlüter et al. 1987). It may be difficult to maintain a stable community, thereby affecting wastewater treatment performance and (protein)-product consistency in the non-sterile wastewater environment. This has been reported for microalgae, especially in open, highly loaded systems (Taiganides 1992) and is commonly referred to as ALBAZOD (algae, bacteria, zooplankton and detritus). This community can be very efficient at removing COD, N and P, but may also deteriorate over time (Oswald 1980). Similarly, both syntrophy and competition have been reported for anaerobic systems with PPBs (Izu et al. 2001). However, studies investigating the long-term effects of organic-rich wastewater on the PPB and flanking community are scarce. A previous study with domestic wastewater (Hülsen et al. 2016a), reported a consistent dominance of PPB species in non-sterile wastewater treatment system. Other studies reported direct competition of PPB with sulfate reducing bacteria (SRB) for hydrogen and volatile fatty acids in the presence of sulfate (Gibson 1990), and SRB can outgrow PPB at higher loading rates (Chiemchaisri et al. 2007).

This present study aims to determine and compare the effect of organic rich wastewater on infrared selected PPB and white light selected microalgae and the effect on the community dominance. At the same time, the overall wastewater treatment capacities and the metabolic modes will be determined and compared with focus on the assimilatory removal of COD, N and P and the formation of protein rich

biomass. For this purpose, continuous open aerobic (microalgae) or closed anaerobic (PPB) photobioreactors were operated in comparative mode. Biomass characteristics and product consistency are also assessed in view of applications as a feed or feed additive, or as an organic fertilizer.

2.Material and methods

Poultry processing plants require between 19 and 38 L of water to process a bird and considering 9 billion birds were processed in 2007 in the US alone (Kiepper et al. 2008), this tremendous amount of wastewater is an important potential source of nutrients. Organics in this wastewater are 95% degradable by anaerobic (anaerobic (Chávez P et al. 2005) and aerobic (Rusten et al. 1998)) treatment which is a prerequisite for efficient biomass growth. The direct recovery of proteins from this wastewater has been proposed by several authors (Lo et al. 2005, Avula et al. 2009) and the fact that poultry by products (feather meal, poultry-by-product-meal, hatchery by-products, spent laying hens) are currently utilized for animal feed (El Boushy and van der Poel 2000) make this stream an interesting source for single cell protein production from for agri-industrial wastewaters.

Raw wastewater

Batches of around 60 L wastewater were collected monthly from a poultry-processing facility in Brisbane, Australia. The wastewater was a combination of process water from feather removal and degutting, as well as general cleaning water (e.g. from cleaning of floors). At the facility, the wastewater is usually pre-screened

(3 mm) and directed towards a dissolved air flotation (DAF) unit. The DAF influent was the wastewater sampled and used in this study. After collection, the wastewater was transported in jerry cans at room temperature (<30 min transport time in a air-conditioned car) and immediately stored in a 4 °C cold room. From this storage, wastewater was weekly pumped to an influent tank in the laboratory after discarding the residues from a previous week of operation. An additional sieve (1.0 mm mesh width, regularly cleaned) was used at the suction side of the influent pump to prevent clogging due to solids.

The raw wastewater contained on average 4000 mgTCOD L^{-1}, 200 mgTKN L^{-1} and 35 mgTP L^{-1} with significant variations in different periods. During Period III, TN and TP increased up to >500 mg L^{-1} and 55 mg L^{-1}, whilst TCOD remained stable. The TCOD increased during Period IV. A detailed composition analysis and the average COD, TN and TP concentrations of each period are in supplementary materials (Table S1 and Figure S1) and each period is further described as part of the bioreactor operation. Changes in wastewater composition resulted from the discharge of different streams such as process water from feather removal and degutting (high COD, TN and TP), as well as differences in general cleaning water amounts. The wastewater of the poultry processing factory is not buffered onsite, because large downstream covered anaerobic lagoons have sufficient hydraulic retention (HRT >20 d) to tolerate peak loads within the system.

172 **Inoculum**

173 The continuous anaerobic reactor described below was inoculated with 10 v/v% of
174 PPB from a domestic wastewater enrichment culture (around 1.0 gVS L^{-1}) as
175 described elsewhere (Hülsen et al. 2016b). We note that inoculum from any mixed
176 microbial source, poultry source wastewater would be feasible (Hülsen et al. 2018).
177 Microalgae was isolated from effluent evaporation ponds at a piggery in South
178 Australia, and cultured in 160 mL serum flasks open to the atmosphere, illuminated
179 with a full spectrum at 130 W m^{-2} by two 150 W fluorescence lamps (distance 63 cm)
180 and continuously stirred at 100 rpm in an orbital shaker (Edwards Instrument
181 Company). From existing cultures, domestic wastewater was inoculated with 10
182 v/v% microalgae on a weekly basis to maintain the cultures in the exponential growth
183 phase. 200 mL of the microalgae (1.0 gVS L^{-1}) enrichment culture was used to
184 inoculate the continuous aerobic reactor described below.

185

186 **White light and infra-red light bioreactors (WLR and IRR)**

187 The IRR and WLR were operated in parallel. The IRR set-up used for the PPB
188 operation was described in detail elsewhere (Hülsen et al. 2016a). The WLR set-up
189 was similar, but open to the atmosphere at the surface. Both reactors were operated
190 at room temperature averaging 22 °C for the WLR reactor, and 24 °C for the IRR
191 reactor (standard deviation of 2°C for both). Both reactors were equipped with a

192 submerged flat sheet membrane with 0.45 mm pore size and 0.12 m^2 surface area
193 (Kubota, Osaka, Japan). The operating flux of the membrane was on average 0.6
194 (0.2) L m^{-2} h^{-1} which is considerable lower than critical flux in the order of 10-20 L m^{-2}
195 h^{-1} (Achilli et al. 2011), due to relatively high strength of the feed. The membrane was
196 mechanically cleaned every 14 d without backwashing.

197 Figure 1 provides a schematic set-up and pictures of both bioreactors. Air was
198 introduced at 6 L min^{-1} (oxygen supply capacity of approx. 240 mgO_2 L^{-1} h^{-1}
199 assuming coarse bubble aeration and a standard transfer efficiency of 3% m^{-1}) with
200 an air compressor (KNF Neuberger Laboport N86KT.18) to mix the WLR and to
201 supply CO_2 and O_2. Given the COD consumption of 60 mg L^{-1} h^{-1}, k_La could be
202 determined to be approximately 15 h^{-1} based on the 1st order model of
203 (Tchobanoglous et al. 2003). Additional oxygen was supplied via photosynthesis,
204 which was estimated at 1000mg O_2 m^2 d^{-1} (or 2.8 mg L^{-1} h^{-1}) at 31 W m^2, at 3%
205 quantum yield and 0.1 mole O_2 per mole photons ($2.46x10^5$ J $mole^{-1}$ (PAR))
206 (Cuaresma et al. 2009)). The WLR was illuminated from one side with a full spectra
207 150 W fluorescence lamp (Clamp Flood Light, Nelson Lighting, Tonbridge, UK). The
208 closed (anaerobic) IRR was illuminated with three IR LED lamps (24 W each, IR 96
209 LED Illuminator for Night Vision Camera, St. Louis, MO, USA) with a spectral
210 distribution of 800-900 nm, and a peak at 850 nm.

211 The spatial distribution of the irradiance was determined for both reactors by taking
212 various measurements along the irradiated surface using a spectroradiometer
213 (stellarnet blue wave spectroradiometer, Warsash Scientific, Australia). Fifty scans
214 were taken and averaged for each point on the surface. The average irradiance of
215 the WLR was 170 W m^{-2} (340-1110 nm) or 31 W m^{-2} of photo active radiation (PAR;

400-700 nm), which is equivalent to 153 µE m^{-2} s^{-1} (1W m^{-2} = 4.94 mol m^{-2} s^{-2} for PAR (Doucha and Lívanský 2009)). This irradiance intensity is common for low intensity indoor illumination of microalgae growth reactors to provide healthy growth that is not photo-inhibited (Chini Zittelli et al. 2000, Mirón et al. 2003). The average irradiance of the IRR was 18.7 W m^{-2} (IR, 750-900 nm), realised by adjusting the distance from emitter to reactor. The spatial distribution and outputs of the lamps are presented in the supplementary materials (Figure S2 and S3).

The attenuation of light in the WL and IR reactors were determined using the Beer-Lambert law. The attenuation for different concentrations of biomass were calculated and plotted. For this analysis, the wavelength for the WL reactor was assumed to be a mean of 590 nm, and 850 nm was assumed as the mean wavelength for the IR reactor. The details of the calculations are presented in the supplementary materials (Figure S10).

The bioreactors were operated for 243 days, which was split into five periods; Period I (01-75 d), Period II (76-143 d), Period III (144-174 d), Period IV (175-226 d) and Period V (227-243 d) During Period I, the bioreactors were operated at 2.0 d HRT, and this was shortened to and maintained at 1.0 d HRT until the end of the tests (Period II-V). The major differences between each period were the wastewater compositions, with low COD, N and P during Period I and II, high TN and TP during period III and high COD, TN and TP during period IV and conditions similar to period I and II during period V (fluctuations caused by grab sampling, also see Figure S1 for comparative influent concentrations). Analyses were generally done 3 times weekly. Contents were regularly withdrawn to control the SRT around 2.0 d and 3.0 d in the IRR and WLR to support PPB and microalgae growth at high influent COD concentrations following Ogbonna et al. (2000). The VLRs of both reactors increased

241 from 2.0 to 3.0 gTCOD $L^{-1}d^{-1}$ (Period I-III) and peaked during Period IV at around 9.3
242 gTCOD $L^{-1}d^{-1}$ where TN and TP VLRs were proportional. A summary of the
243 experimental design, including the volumetric loading rates (VLR) for TCOD, TN and
244 TP, as well as the solids loading rate (SLR), are presented in Figure S4.

245 The reactors were completely mixed with air (WLR) or headspace gas (IRR, recycled
246 from the headspace to the base of the reactor). The reactor contents (denoted below
247 as sludge out) were sampled from the middle of the reactor, and these samples were
248 assumed to be representative of the well-mixed reactor contents. Membrane filtrate
249 was also regularly sampled, denoted as “reactor effluent” below. These samples
250 were analysed for TCOD, TKN, TP, NH_4-N,NO_x-N, NO_2-N, PO_4-P, Sulfur species,
251 metals and VFAs by the analytical methods described below. The biofilm from the
252 membrane and reactor walls was regularly removed and re-suspended in both
253 systems (once per week).

254

255 **Analytical methods**

256 TCOD and SCOD were determined by COD cell tests (Merck, 1.14541.0001,
257 Darmstadt, Germany). Dissolved NH_4-N, NO_x-N, NO_2-N and PO_4-P were determined
258 by a QuikChem8000 FIA (Hach Company, Loveland, USA). Temperature and pH
259 were measured using a TPS Minichem temperature and pH set-up (Brendale, QLD,
260 Australia). TSS and VSS and TS/VS were determined according to Standard
261 Methods (APHA. 1998). Soluble and total Kjeldahl nitrogen (TKN) and total

phosphorus (TP) were determined using sulfuric acid, potassium sulfate and copper sulfate catalyst in a block digestor (Lachat BD-46, Hach Company, Loveland, CO, USA) (Patton and Truitt 1992). Elemental analysis was performed by inductively coupled plasma optical emission spectrometry (ICP-OES) after 10 % nitric acid digestion (Perkin Elmer with Optima 7300 DV, Waltham, MA, USA). Protein analysis was estimated by calculation based on NH_4-N and TKN content following the approach described by Eding et al. (2006). Additionally, amino acid contents were analysed externally by the Australian Proteome Analysis Facility (Sydney, Australia). For the amino acid quantification, samples were hydrolysed with 6M HCl at 110 °C for 24 hours and then analysed using WaTERS aCCqtAG Ultra chemistry on a Waters Acquity UPLC for high sensitivity amino acid analysis. All soluble constituents analysed during the reactor operations were determined after filtering with a 0.45 µm membrane filter (Millipore, Millex®-HP, Merck Group, Darmstadt, Germany). Irradiance (W m^{-2}) and wavelength profile were measured with a UV-VIS & NIR light sensor as described above (stellarnet blue wave spectroradiometer, Warsash Scientific, Australia). Gas samples (CO_2 and CH_4) were analysed by GC (2014 Shimadzu, Kyoto, Japan) with thermal coupled detector (TCD) (Tait et al. 2009).

DNA extraction and Amplicon sequencing

Genomic DNA was extracted from the samples by FastSpin for Soil Kit (MP-Biomedicals, Santa Ana, CA, USA) according to the manufacturer's protocol. DNA of each sample was provided to Australian Centre for Ecogenomics (ACE) for 16S Amplicon sequencing by Illumina Miseq Platform, using universal primer pair 926F (5'-AAACTYAAAKGAATTGACGG-3') and 1392wR (5'-ACGGGCGGTGWGTRC-3') primer sets (Engelbrektson et al. 2010).

Raw paired reads was first trimmed by Trimmomatic (Bolger et al., 2014) to remove short reads (less than 190bp) and low quality (lower than Phred-33 of 20). The trimmed paired reads was then assembled by Pandaseq (Masella et al. 2012) with default parameters. The adapter sequences were removed by FASTQ Clipper of FASTX-Toolkit (Pearson et al. 1997). The joined high quality sequences was analysed by QIIME v1.8.0 (Caporaso et al. 2010) using open-reference operational taxonomic unit (OTU) picking strategy by uclust (Edgar 2010) at 3% phylogenetic distance and assigned taxonomy by uclust against Silva database (128_release) (Quast et al. 2012). OTUs with only one or two reads were filtered from the OTUs table by command filter_otus_from_otu_table.py in QIIME. Then filtered OTUs table was normalised to 9000 reads per sample using package phyloseq (McMurdie and Holmes 2013) in R (version 3.2.1; R core team, 2015). Normalised OTUs table was then summarised to genus level by command summarize_taxa.py in QIIME.

Data processing and statistical analysis

Cumulative mass balances were performed on TCOD, TN and TP, as well as K and Mg to determine losses and recovery potential. This analysis used Eq. (1), where *Xi* indicates the concentration *X* of the component *i*. Mass loadings were calculated using hydraulic daily influent flow multiplied by measured mass concentration. Calculations for which daily data was missing in the effluent or sludge, linear interpolation (interp1 command in Matlab) of data measured on the days before and after were utilized. The COD capture efficiency was calculated with the interpolated loads, considering the particulate COD (TCOD-SCOD) of the withdrawn sludge over the TCOD influent load over time.

$$Xi_{recovery}(\%) = \sum_{i=1} \frac{Xi_{effluent}(g\ d^{-1}) + Xi_{sludge\ out}\ (g\ d^{-1})}{Xi_{influent}\ (g\ d^{-1})}\ x100 \quad (1)$$

$$\text{COD capture efficiency}\ (gCOD\ gCOD^{-1}) = \sum_{i=1} \frac{SolidCOD_{sludge\ out}\ (g\ d^{-1})}{TCOD_{influent}\ (g\ d^{-1})} \quad (2)$$

Crude protein recovery per tonne COD_{in} =

$$\frac{TCODin\ (kg)}{\left(\frac{TCOD}{VS}\right)} \times\ \eta(COD)\ (\%) \times Y(kgCOD\ kgCOD^{-1}) \times CP\ content\ (kgCP\ kgVS^{-1}) \quad (3)$$

Measurement results for inputs are presented below as averages, and variability in inputs are expressed as standard deviations in time-series measurements, given as $\bar{X}(s_{X_i})$, where $\bar{X}$, is the average value for the data X_i, and s_{X_i} is the corresponding standard deviation. Measurement results for outputs and calculated parameters are presented below as average values, with uncertainty expressed as uncertainty in the mean value based on a two-tailed t-test at the 95% confidence level (5% significance threshold) and with an appropriate number of degrees of freedom. Thus values are given below as $\bar{X} \pm E_{\bar{X}}$, where $E_{\bar{X}}$ is the estimated error at the 95% confidence level. The 'Shannon' index of species diversity (H) was determined using $H =$

$-\sum_{i=1}^{n} p_i \ln p_i$ with p_i being the number of reads in an OUT/total number of reads (Spellerberg and Fedor 2003). Shannon evenness (E) was determined using $E = \frac{H}{lnS}$ (S = number of total OTUs in the sample) following (Hill et al. 2003).

3. Results and Discussion

Wastewater treatment performance

Both the WLR and IRR were able to effectively treat the poultry processing wastewater with COD removals (averages calculated between day 20 – day 243) averaging 98.1±0.5 % for the WLR and 92±1.5 % for the IRR. This was with sustained loading rates up to 15 kgCOD $m^{-3}d^{-1}$ and a short HRT of 1.0 d. Effluent TCOD was 110 mg L^{-1} and 296 mg L^{-1}, respectively. The pH averaged 7.0 for the WLR and 7.5 for the IRR with a standard deviation of 0.2 units on both. The two reactors achieved similar TN removals, being specifically 68±6 % for the WLR and 63±5 % for the IRR. Average effluent TN concentrations were 74 mg L^{-1} and 93 mg L^{-1}, respectively. The average TP removal over time (day 20 – day 243) was 26±2.9 % for the WLR and 35.5±3.0 % for the IRR, with final effluent concentrations of 27 mgP L^{-1} and 24 mgP L^{-1}, respectively.

TCOD, TN and TP removal efficiencies changed over time in both reactors, with average removal efficiencies for the various treatment periods being significantly

343 different ($p<0.05$) (Figure 2A-C). Nevertheless, measured TN and TP removals were
344 similar during specific periods (e.g. TN and TP in Period III ($p>0.05$); TP in Period IV
345 ($p>0.05$); TN in Period V ($p>0.05$)), and mostly coincided with changes in influent
346 wastewater composition/strength. The bioreactors showed improved TN and TP
347 removal performance with higher wastewater strength (Period III and IV, Figure 3).
348 The TCOD, TN and TP effluent concentrations time series are shown in Figure S5.

349 The decrease in HRT from 2.0 d to 1.0 d did not significantly affect average TCOD
350 removal performance in either of the two reactors. However, during Period IV with
351 the high COD influent, the removal performance of the WLR was stable whereas the
352 IRR showed a progressive increase of up to 700 mgCOD L^{-1} in the effluent, partly
353 explained by an increase in VFA-COD (Figure S6).

354 The TN removal performance increased with increasing influent TN (up to 785 mg L^{-1})
355 during Period III (Figure 2B), and effluent TN concentrations remained similar to
356 that observed in Periods I and II. This increased N removal was likely achieved by
357 retaining organically bound N in the sludge. However, TN effluent concentrations
358 decreased during Period IV, despite increased COD influent concentrations during
359 this Period, also indicating assimilative N removal. Average N removal was not
360 significantly different between periods III and V. However, the main nitrogen species
361 in treated effluent from the WLR was nitrate, as opposed to ammonium in the IRR
362 effluent (Figure S7), suggesting that fundamentally different N removal pathways

363 were giving similar removal performance. Excess nitrification also caused the TN
364 efficiency drop in the WLR around day 140, which resulted from a programmable
365 logic controller interruption, stopping the influent pump but not the air supply.

366 Total phosphorus removal was not affected by the HRT change (Period I and II). The
367 TP increase in the influent during Period III translated into a higher TP concentration
368 in the effluent (Figure S1). However, TP concentration in the effluent decreased
369 during Period IV despite higher COD influent concentrations, thought to be due to
370 assimilative TP removal as described for TN above.

371 Overall, discharge limits to e.g. surface waters were not achieved in the one-step
372 treatment but, depending on legislation, the application as irrigation water might be
373 feasible.

374 **COD, N and P recovery**

375 Mass balance over 124 days of operation for the WLR showed that a major fraction
376 of the COD was lost (64±1.6 %), likely dissipated via respiration to CO_2. At the same
377 time, 25±1.4 % of the nitrogen (N) was lost, probably by stripping of ammonia or
378 nitrification/denitrification (García et al. 2000). Mass balances for phosphorus (P) and
379 potassium (K) indicated closure at 103.4±0.4 % and 107.1±8.0 %. Overall, 32.7±2.0
380 % of COD, 36.2±0.7 % of N and 28.6±0.4 % of P in the influent was
381 assimilated/retained as biomass/product in the WLR.

With the IRR, the recovery of TCOD (via VFAs-COD) to biomass of (48.7±1.0 %), N (45.7±2.0 %) and P (44±3.0 %) was higher than in the WLR. Overall N and K balances on the IRR also showed reasonable closure at 100.7±1.2 % and 97.9±1.9 %, respectively. Off-gas production (IRR), measured a gas flow (tipping bucket gas meter) of around 1.2 L over the entire reactor operation with minor amounts of methane 2.9 % CH_4, 2.8 % CO_2 (standard deviation of 1.5 % on both), and negligible hydrogen (~0.01 %). This represents a negligible COD loss through methanogenesis. A significant proportion of P was lost, with only 80±4.0 % of the influent load being recovered in effluent or sludge. This loss of P is likely due to P-precipitation with Ca and Mg, and settling of precipitates to the bottom of the reactor, despite the vigorous gas mixing applied during the tests (partly caused by the elevated pH). Detailed progressive cumulative TCOD, N and P mass balances are presented in the supplementary materials (Figure S8 and S9) for reference.

Biomass characteristics

The biomass characteristics of both reactors were well in line with literature values reported by Shipman et al. (1975) (PPB) and Anupama and Ravindra (2000) (microalgae) as well as a more recent comparison between microalgae, fungi, bacteria and PPB (Puyol et al. 2016). Biomass characteristics were affected by the influent composition of each operation period. However, comparing the crude protein

(calculated from particulate TKN) content of periods I and II (over 143 d) shows that both reactors generated biomass with >0.6 gCrude Protein (CP) gTS^{-1} (compared to 0.6-0.65 and 0.45-0.65 gCP gTS^{-1} (Puyol et al. 2016)) with average biomass yields for the IRR and the WLR of 0.5±0.1 gCOD $gCOD^{-1}$ and 0.3±0.2 gCOD $gCOD^{-1}$ (extracted from TCOD sludge fractions in Figure 4). The average biomass yield of the IRR was up to 60 % higher than that of the WLR. At the same time, the N and P content and TCOD/VS ratios of the biomass, were essentially the same for both reactors (1.4 % P and 10 % N and 1.5±0.1 TCOD/VS, data not shown).

The total protein content according to the amino acid analysis was higher in the IRR biomass (460 mg amino acid gTS^{-1} or 502 mg gVS^{-1}) than in the WLR biomass (380 mg gTS^{-1} or 390 mg gVS^{-1}) (also well in line with above cited literature) (Figure 5). Amino acid composition was only considered during Period I and II (n=5), because particulate matter and increased crude protein (CP) content in higher strength influent wastewater of later operating periods. The CP influent concentration during these periods increased to >3.4 g L^{-1} which means at least part of this protein is measured together with the PPB protein in the reactor. Anaerobic protein hydrolysis and fermentation to VFA, CO_2 and NH_4-N is commonly not complete due to thermodynamic, stoichiometric (Ramsay and Pullammanappallil 2001) and kinetic effects (Christ et al. 2000). When harvesting the reactor biomass, the total protein measurements also contained poultry processing wastewater proteins that are

422 naturally parts of the solids. This potentially interfered with the PPB protein profile
423 during Period II and IV. This would need to be considered if microbial protein were
424 the goal, with waste chicken fraction being acceptable (e.g., for fish feed) (Samocha
425 et al. 2004), or better solids removal would be required.

426 Metal contaminants were also tested, and were generally low with Cd, Ni and As
427 being undetectable (<0.00 mg/kg), Pb and Cu being <0.4 mg/kg, and Zn being <1.0
428 mg/kg, which would allow the global application as fertilizer and animal feed in China
429 and Canada, following the limits values summarised by Wang et al. (2017). There
430 were no systematic differences between the two products. This offers no barriers to
431 application of the final product as feed or fertilizer. Further information is provided in
432 Table S2.

433 **Microbial analysis**

434 The microbial analysis of the WLR showed that photosynthetic organisms were
435 present over the reactor operation period, but did not dominate (Figure 6). The
436 highest relative abundance of phototrophic organisms was <20% (93 d), with
437 *Chlorella* being the most abundant genus (16%, at 93 d). A variety of cyanobacteria
438 (*Chorophyta*), green non-sulfur (*Chloroflexi*), green sulfur (*Chlorobi*) and purple
439 phototrophic bacteria (*Blastochloris*, *Rhodobacter*, *Rubivax*, *Thiocapsa*) were
440 present as flanking phototrophs, at less than 3% relative abundance. The flanking
441 PPB can act as aerobic anoxygenic phototrophs (Yurkov and Beatty 1998),

442 contributing to COD and nitrogen removal at high dissolved oxygen previously
443 reported (Lu et al. 2017).

444 The WLR non-phototrophic community was generally comprised of common
445 (facultative) aerobic heterotrophs, frequently detected in aerobic systems including
446 *Comamonadaceae* (*Acidovorax*, *Hydrogenphaga*, *Schlegelella*), *Xanthobacteraceae*
447 (*Xanthobacter, Dokdonella*) (4-12 % relative abundance), *Chitinophagaceae* (0.17-
448 38 %) and *Ellin6075* (0.04-3 %) (Cao et al. 2014, Oren 2014, Rosenberg 2014) but
449 shifted over time. This distribution also explains the neutral pH of the WLR were
450 algae consumed inorganic carbon originating from organic carbon conversion by
451 heterotrophs. The Shannon evenness index (E) was stable around 0.67, indicating
452 very small changes of the diversity in the WLR community over time. This indicates,
453 white light did not create a selection advantage for microalgae, which would have
454 resulted in dominance and a subsequent decreased equitability over time.

455 In contrast to the WLR, the evenness index for the IRR decreased from 0.6 to 0.4 in
456 the IRR over time (Figure 6). This indicates reduced diversity due to increased
457 dominance of PPB driven by IR in combination with increased influent COD which
458 also resulted in increased VFA-COD concentrations in the reactor (>200 mg VFA-
459 COD L^{-1} as show in Figure S6). The VFA-COD reactor concentration had significant
460 effect of the relative abundances of PPB over time ($p = 0.022$). The most abundant
461 PPB species was *Rhodopseudomonas palustris* (up to 50 %), followed by

462 *Rhodobacter sphaeroides* (up to 23 %) and a variety of flanking PPBs including
463 Rubrivivax *sp*. *Rhodocyclus sp*, *Blastochloris sp*. *Allochromatium* and *Thiocapsa sp.*
464 (each max 1.0 %). The community shifted towards PPB dominance whereby
465 changing influent conditions, and particularly high influent COD (Period IV, day 201),
466 favoured *Rps. palustris* at day 201.

467 The flanking community was comprised of a diverse mix of common representatives
468 in anaerobic digesters, including a variety of *Bacteroidetes (Dysgonomonas,* 10.7 %;
469 201 d), *Proteobacteria* (*Moraxellaceae,* 11 %; 168 d) and *Firmicutes*
470 (*Carnobacterium,* 8.6 %; 159 d) that degrade oligomers such as fats, carbohydrates
471 and proteins into smaller subunits such as VFAs and NH_4-N (Wang et al. 2014)
472 utilized by PPBs to produce protein rich biomass. Interestingly, methanogens,
473 specifically fast growing hydrogenotrophs did not become established and were
474 present at low relative abundance of <3.0 % at day 168 and generally below 1.0 %,
475 aligning with the minimal methane production observed.

476

477 **Comparing WL and IR reactors**

478 *Performance.* Both bioreactor systems were able to simultaneously remove organic
479 matter, N and P from poultry processing wastewater at reasonable loading rates
480 (average 4.0 kg m^{-3} d^{-1}) and reasonable HRT (1.0 d). The comparison showed

similar overall removal performance by the two reactors with 42 % less effective irradiance in the IRR (comparing 18 W m^{-2} with 31 W m^{-2} PAR). When considering emitted illumination, the savings increases to 90% comparing IR irradiance (18 W m^{-2}) with a full spectra (170 Wm^{-2}). We acknowledge that efficient UV-VIS LEDs would more efficiently deliver the same PAR output, but will never approach the efficiency of IR illumination for artificial illumination. We further note, light attenuation is strongly impacted by biomass concentration and wavelength. White light is not attenuated in pure water over 50 mm, while IR light is attenuated by 20 % (Fig S10). However, even at 0.2 gVSS L^{-1}, IR light is less attenuated than WL (90 % over 50 mm for IR vs 95 % for WL). Penetration depth for both, in the presence of biomass is comparably small, being 5 mm for WL at 2.0 gVSS L^{-1} vs 10 mm for IR. This emphasises the importance of mixing and hydrodynamics in delivering the microorganisms to the irradiated surface, as opposed to delivering photons to the microorganisms (Bitog et al. 2011).

Microbial community and yield. As expected, in the WLR (aerobic), microalgae were not the main mediator for wastewater treatment and biomass assimilation (Hülsen et al. 2018). This contrasts with previous reports, where similar intensities to the ~30 W m^{-2} PAR were able to support non-photo inhibited microalgae (*Nannochloropsis, Chlorella, Spirulina*) growth, even at peak growth rates (Sforza et al. 2012, Simionato et al. 2013, Gouveia and Oliveira 2009). The applied SRT was above the minimum

501 for *Rhodobacter sphaeroide*s, *Chlorella sorokiniana* and *Spirulina platensis*
502 (Ogbonna et al. 2000). This is further supported by specific growth rates of *Chlorella*
503 *sorokiniana* and *Leptolyngbya* (0.32 d^{-1}on glucose (Wan et al. 2011) or 0.24 – 0.27
504 on bicarbonate (Janssen et al. 1999)); 0.40 d^{-1} on CO_2 (van der Grinten et al. 2005)).
505 The key difference is likely due to two factors; (i) the air supplied through membrane
506 scouring and mixing, and (ii) the complex wastewater source. Therefore treatment of
507 this type of wastewater always results in ALBAZOD referring to the diverse
508 community of algae, bacteria, zooplankton and detritus (Hargreaves 2006).
509 Population shifts in open microalgal ponds due to grazing and population losses are
510 density depended whether fed with wastewater or pure substrates (Day 2013), and
511 observed in pilot and large scale ponds in a sophisticated 10-year study in Singapore
512 (Taiganides 1992). This mixed (bacterial dominated) community of the WLR
513 resulted in a similar yield (0.34±0.01 gCOD $gCOD^{-1}$) compared to yields obtained in
514 conventional aerobic systems which have up to 20 d SRT (at 20 °C ~ 0.3-0.4 gCOD
515 $gCOD^{-1}$ (Tchobanoglous et al. 2003)) as well as high rate activated systems with
516 SRT of 2 d (Jimenez et al. 2015).
517 In contrast, the measured yields for the IRR (0.5±0.01 gCOD $gCOD^{-1}$) fell within in
518 the broad range of values reported in the literature for mostly axenic PPB cultures
519 treating various agri-industrial wastewaters (Tuna condensate + Shrimp blanching
520 water: 0.24 (Prasertsan et al. 1993), mandarin orange peel: 0.83 (Sasaki et al. 1991)

521 and Sago starch processing (decanter) 0.59 gCOD $gCOD^{-1}$ (Getha et al. 1998)). The
522 measured yields were lower than that reported for a mixed PPB community treating
523 domestic wastewater (~1.0 gCOD $gCOD^{-1}$) (Hülsen et al. 2016a) which is likely
524 attributed to the decreased relative PPB abundance (~50 % compared to >80 %)
525 induced by high poultry wastewater influent COD and the need of e.g. fermenting
526 bacteria to transform the oligomers into VFAs. Despite the anaerobic conditions,
527 anaerobic protozoa can also graze and pose a potential threat to PPBs (Schulz et al.
528 1990) and further research is required to clarify these effects for large-scale
529 production systems.
530
531 *Amino acid profile.* In comparison with fishmeal (FAO 1970), the overall protein level,
532 and hence amino acid content was lower in both reactors. Based on a crude protein
533 content of 60 %, around 190 kg (IRR) and 140 kg (WLR) of crude protein could be
534 recovered per tonne of influent COD per day from poultry processing wastewater
535 (calculated following Eq.3). The influence of non-degraded influent protein on the
536 biomass characteristics could be reduced by buffering to avoid peak loads, which
537 would also allow for a degree of pre-acidification. In terms of the single cell protein
538 (SCP) quality, a stable microbial community is advantageous as product quality,
539 consistency and therefore potential value are likely improved. As such, PPB can
540 produce a more consistent product when treating organic-rich wastewaters, because

541 of the dominance by PPB as compared to an ALBAZOD based system. However, for
542 the overall biomass protein content and recovery, this would be secondary, because
543 protein residues already present in the raw wastewater could contribute to the
544 harvested product. We note that e.g. in the European Union (EU 2002), high grade
545 feed mixtures are downgraded to lower categories if co-mixed animal by-products
546 (e.g. SCP produced on wastewater) have a low grade (Woodgate and Van Der Veen
547 2004) which likely impacts the SCP value, but does not prohibit the application in
548 general. In terms of price, this may lead to a preference for non-animal derived
549 wastewaters, e.g. sugar mill/stillage, and these aspects require further clarification.
550 However, we note that e.g. sugar mill wastewater also has drawbacks as it a) does
551 not contain sufficient macro and micronutrients, b) production is seasonal, and c)
552 wastewater is recycled within the process throughout the production season
553 (personal communication with the management of the Condong Sugar Mill, Australia
554 and stream inventory campaign, data not shown).
555

556 **Significance for agricultural industrial wastewater treatment**

557 Treatment of poultry processing wastewater in the present study did not achieve
558 typical surface water discharge limits (TCOD <100, TN<10 and TP<1.0 mg L^{-1}), and
559 barely achieved irrigation water limits. However, the results did show that, with
560 sufficient influent COD, significant amounts of N and P can be assimilated at

561 average volumetric loads of 4.0(3.1) kgTCOD m^3 d, 0.3(0.19) kgTN m^{-3} d^{-1} and
562 0.03(0.01) kgTP m^{-3} d^{-1}. Instead of producing low value biogas and operating at low
563 biomass yields without nutrient recovery, an SCP concept could increase microbial
564 yields as well as upgrade nutrients to valuable products. Rather than aiming to
565 reduce sludge production as in traditional wastewater treatment, a SCP concept
566 aims for maximum recovery of COD, N and P, which are then considered to be
567 valuable resources. There is an increasing interest in value add products and the
568 upgrading of e.g. low value methane (Strong et al. 2016). In this context, Burgess et
569 al. (2015) has determined that on a mass load basis (from domestic wastewater
570 input loads), the value of microbial biomass is approximately 3 times that of biogas.
571 However, this applied a relatively low value of AUD 0.2 kg^{-1} to microbial biomass.

572 The results showed that IRR has the potential to produce around 500 kg of protein
573 rich biomass-COD per tonne of influent COD (assuming the presence of adequate
574 amounts of N and P). This achieves a significantly larger recoverable fraction in the
575 wastewater treatment as compared to conventional anaerobic (methanogenic)
576 technologies. Although, the amino acid content of the recovered product was lower
577 than fishmeal, the data suggested that at least a fraction of fishmeal could be
578 substituted with the biomass product where the value of fishmeal is between 1400-
579 1800 USD $tonne^{-1}$ (Asche et al. 2013) which would result in 10 times higher values
580 compared to biogas following Burgess et al. (2015). Ultimately, the value of the

biomass has to be determined in feed trials, but the relevant literature has already suggested the general applicability of phototrophic bacteria grown in axenic cultures on artificial medium (Banerjee et al. 2000, Azad et al. 2002, Kim and Lee 2000). However, for axenic cultures grown on artificial medium, the production costs would likely exceed the product value and render the protein source economical unfeasible, except for exceptionally high value niche applications. This has been addressed for microalgae and biofuel production (Slade and Bauen 2013) where the utilisation of wastewater has the potential to reduces the overall production costs (Park et al. 2011). We note, axenic cultures are not considered to be directly applicable to wastewater treatment and comparisons and life cycle analysis for PPB biomass are not available in the literature yet.

The IRR could produce a PPB dominated culture, with irradiance reduced to 8.0 kWh m^{-3} (18 W m^{-2} at 18 m^2 m^{-3}). Based on a volumetric removal rate of up to 3.6 kgCOD m^{-3} d^{-1} (90% COD removal) and a yield of 0.5 kgCOD $kgCOD^{-1}$ with 1.6 gTCOD gVS^{-1}, the net radiation energy required would then be around 7 kWh per dry tonne (25 GJ). In this case, one tonne of PPB contains up to 22.2 GJ, chemical energy largely sourced from input COD (based on 3.86 kWh $kgCOD^{-1}$ (McCarty et al. 2011)).

The potential product value in conjunction with the production costs will determine the future payback period and real world applications of a SCP mediated system, such as PPB technology. In parallel, resource recovery will become an economic

601 driver in cyclic wastewater treatment and new technologies will be required in place
602 of conventional cradle to grave solutions (Matassa et al. 2015).

603 4.Conclusion

604 The comparison of the IRR (PPB) and the WLR (ALBAZOD) showed that both
605 systems removed around 90 % COD, 90 % TN and 40 % TP although the underlying
606 processes were fundamentally different. The anaerobic PPB reactor outperformed
607 the aerobic system in terms of organics and nutrients assimilation and potential
608 resource recovery as protein rich biomass. The biomass of both systems might serve
609 as a future feed or feed additive where the IRR was clearly dominated by PPB (>55
610 %) compared to <20 % of microalgae in the WLR, which can result in more
611 consistent product quality and value over time. WLR systems may be more effective
612 where natural light is preferred, or where nutrient removal is more important than
613 resource recovery.

614

615 **Acknowledgments**

616 We gratefully acknowledge Dr Beatrice Keller and Nathan Clayton from the
617 Advanced Water Management Centre for assistance with analytical measurements.
618 We also thank Dr Nicola Angel from the Australian Centre for Ecogenomics for
619 assistance with 16S rRNA amplicon sequencing, Dr Yang Lu for microbial support

620 and Dr Andrew Ward for assisting with the microalgae set-up. This work was funded
621 by Australian Pork Limited and the Department of Agriculture and Forestry, Australia
622 (Project No: 2014/534.05) as part of its Rural R&D for Profit programme.

623 5.References

624 Achilli, A., Marchand, E.A. and Childress, A.E. (2011) A performance evaluation of
625 three membrane bioreactor systems: aerobic, anaerobic, and attached-growth.
626 Water Science and Technology 63(12), 2999-3005.

627 Adessi, A. and De Philippis, R. (2014) Photobioreactor design and illumination
628 systems for H2 production with anoxygenic photosynthetic bacteria: A review.
629 International Journal of Hydrogen Energy 39(7), 3127-3141.

630 Angenent, L.T., Karim, K., Al-Dahhan, M.H., Wrenn, B.A. and Domíguez-Espinosa,
631 R. (2004) Production of bioenergy and biochemicals from industrial and agricultural
632 wastewater. Trends in Biotechnology 22(9), 477-485.

633 Anupama and Ravindra, P. (2000) Value-added food:: Single cell protein.
634 Biotechnology Advances 18(6), 459-479.

635 APHA. (1998) Standard Methods for the Examination of Water and Wastewater. 20th
636 ed, American Public Health Association, Washington, DC, USA.

637 Asche, F., Oglend, A. and Tveteras, S. (2013) Regime Shifts in the Fish
638 Meal/Soybean Meal Price Ratio. Journal of Agricultural Economics 64(1), 97-111.

639 Avula, R.Y., Nelson, H.M. and Singh, R.K. (2009) Recycling of poultry process
640 wastewater by ultrafiltration. Innovative Food Science & Emerging Technologies
641 10(1), 1-8.

642 Azad, S.A., Chong, V.C. and Vikineswary, S. (2002) Phototrophic bacteria as feed
643 supplement for rearing Penaeus monodon larvae. Journal of the World Aquaculture
644 Society 33(2), 158-168.

645 Azov, Y., Shelef, G. and Moraine, R. (1982) Carbon limitation of biomass production
646 in high-rate oxidation ponds. Biotechnology and Bioengineering 24(3), 579-594.

647 Banerjee, S., Azad, S., Vikineswary, S., Selvaraj, O. and Mukherjee, T. (2000)
648 Phototrophic bacteria as fish feed supplement. Asian Australasian Journal of Animal
649 Sciences 13(7), 991-994.

650 Batstone, D.J., Hülsen, T., Mehta, C.M. and Keller, J. (2015) Platforms for energy
651 and nutrient recovery from domestic wastewater: A review. Chemosphere 140, 2-11.

652 Berberoglu, H. and Pilon, L. (2007) Experimental measurements of the radiation
653 characteristics of Anabaena variabilis ATCC 29413-U and Rhodobacter sphaeroides
654 ATCC 49419. International Journal of Hydrogen Energy 32(18), 4772-4785.

655 Bitog, J.P., Lee, I.B., Lee, C.G., Kim, K.S., Hwang, H.S., Hong, S.W., Seo, I.H.,
656 Kwon, K.S. and Mostafa, E. (2011) Application of computational fluid dynamics for
657 modeling and designing photobioreactors for microalgae production: A review.
658 Computers and Electronics in Agriculture 76(2), 131-147.

659 Borowitzka, M.A. (1999) Commercial production of microalgae: ponds, tanks, tubes
660 and fermenters. Journal of Biotechnology 70(1), 313-321.

661 Brown, M.R. (1991) The amino-acid and sugar composition of 16 species of
662 microalgae used in mariculture. Journal of Experimental Marine Biology and Ecology
663 145(1), 79-99.

664 Buitrón, G., Figueroa-González, I. and Quijano, G. (2018) Kinetic characterization of
665 Scenedesmus quadricauda under low irradiation conditions. Journal of Chemical
666 Technology & Biotechnology 93(3), 842-848.

667 Burgess, J., Batstone, D., Muster, T. and Pamminger, F. (2015) Wastewater: An
668 untapped resource. Canberra: Australian Academy of Technological Sciences and
669 Engineering (ATSE).

670 Cao, J., Lai, Q., Liu, Y., Li, G. and Shao, Z. (2014) Ottowia beijingensis sp. nov.,
671 isolated from coking wastewater activated sludge, and emended description of the
672 genus Ottowia. International journal of systematic and evolutionary microbiology
673 64(3), 963-967.

674 Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello,
675 E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T.,
676 Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D.,
677 Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann,
678 J., Yatsunenko, T., Zaneveld, J. and Knight, R. (2010) QIIME allows analysis of high-
679 throughput community sequencing data. Nat Meth 7(5), 335-336.

680 Chávez P, C., Castillo L, R., Dendooven, L. and Escamilla-Silva, E.M. (2005) Poultry
681 slaughter wastewater treatment with an up-flow anaerobic sludge blanket (UASB)
682 reactor. Bioresource Technology 96(15), 1730-1736.

683 Chiemchaisri, C., Jaitrong, L., Honda, R., Fukushi, K. and Yamamoto, K. (2007)
684 Photosynthetic bacteria pond system with infra-red transmitting filter for the
685 treatment and recovery of organic carbon from industrial wastewater, pp. 109-116.

686 Chini Zittelli, G., Pastorelli, R. and Tredici, M.R. (2000) A Modular Flat Panel
687 Photobioreactor (MFPP) for indoor mass cultivation of Nannochloropsis sp. under
688 artificial illumination. Journal of Applied Phycology 12(3), 521-526.

689 Chowdhury, A.J.K., Zakaria, N.H. and Abidin, Z.A.Z. (2016) Phototrophic Purple
690 Bacteria as Feed Supplement on the Growth, Feed Utilization and Body
691 Compositions of Malaysian Mahseer, Tor tambroides Juveniles. Sains Malaysiana
692 45(1), 135-140.

693 Christ, O., Wilderer, P. and Faulstich, M. (2000) Mathematical modeling of the
694 hydrolysis of anaerobic processes. Water Science and Technology 41(3), 61-65.

695 Corominas, L., Foley, J., Guest, J.S., Hospido, A., Larsen, H.F., Morera, S. and
696 Shaw, A. (2013) Life cycle assessment applied to wastewater treatment: State of the
697 art. Water Research 47(15), 5480-5492.

698 Craggs, R.J., Heubeck, S., Lundquist, T.J. and Benemann, J.R. (2011) Algal biofuels
699 from wastewater treatment high rate algal ponds. Water Science and Technology
700 63(4), 660-665.

701 Cuaresma, M., Janssen, M., Vílchez, C. and Wijffels, R.H. (2009) Productivity of
702 Chlorella sorokiniana in a short light-path (SLP) panel photobioreactor under high
703 irradiance. Biotechnology and Bioengineering 104(2), 352-359.

704 Day, J.G. (2013) Grazers: the overlooked threat to the sustained production of future
705 algal biofuels. Biofuels 4(5), 459-461.

706 Doucha, J. and Lívanský, K. (2009) Outdoor open thin-layer microalgal
707 photobioreactor: potential productivity. Journal of Applied Phycology 21(1), 111-117.

708 Edgar, R.C. (2010) Search and clustering orders of magnitude faster than BLAST.
709 Bioinformatics 26(19), 2460-2461.

710 Eding, E.H., Kamstra, A., Verreth, J.A.J., Huisman, E.A. and Klapwijk, A. (2006)
711 Design and operation of nitrifying trickling filters in recirculating aquaculture: A
712 review. Aquacultural Engineering 34(3), 234-260.

713 El Boushy, A.R.Y. and van der Poel, A.F.B. (2000) Handbook of Poultry Feed from
714 Waste: Processing and Use, pp. 90-152, Springer Netherlands, Dordrecht.

715 Engelbrektson, A., Kunin, V., Wrighton, K.C., Zvenigorodsky, N., Chen, F., Ochman,
716 H. and Hugenholtz, P. (2010) Experimental factors affecting PCR-based estimates of
717 microbial species richness and evenness. ISME J 4(5), 642-647.

718 EPA. (2000) Environmental Guidelines: Use and Disposal of Biosolids Products.
719 New South Wales (NSW) EPA 97/62.

720 EU (2002) Regulation (EC) No 1774/2002 of the European Parliament and of the
721 Council of 3 October 2002 laying down health rules concerning animal by-products
722 not intended for human consumption

723 FAO (1970) Amino-Acid Content of Foods and Biological Data on Proteins. FAO
724 United Nations, Rome.

725 Foley, J., de Haas, D., Hartley, K. and Lant, P. (2010) Comprehensive life cycle
726 inventories of alternative wastewater treatment systems. Water Research 44(5),
727 1654-1666.

García, J., Mujeriego, R. and Hernández-Mariné, M. (2000) High rate algal pond operating strategies for urban wastewater nitrogen removal. Journal of Applied Phycology 12(3-5), 331-339.

Getha, K., Vikineswary, S. and Chong, V.C. (1998) Isolation and growth of the phototrophic bacterium Rhodopseudomonas palustris strain B1 in sago-starch-processing wastewater. World Journal of Microbiology and Biotechnology 14(4), 505-511.

Gibson, G.R. (1990) Physiology and ecology of the sulfate-reducing bacteria. Journal of Applied Bacteriology 69(6), 769-797.

Gouveia, L. and Oliveira, A.C. (2009) Microalgae as a raw material for biofuels production. Journal of Industrial Microbiology & Biotechnology 36(2), 269-274.

Hale, G.M. and Querry, M.R. (1973) Optical constants of water in the 200-nm to 200-μm wavelength region. Applied Optics 12(3), 555-563.

Hargreaves, J.A. (2006) Photosynthetic suspended-growth systems in aquaculture. Aquacultural Engineering 34(3), 344-363.

Hill, T.C.J., Walsh, K.A., Harris, J.A. and Moffett, B.F. (2003) Using ecological diversity measures with bacterial communities. FEMS Microbiology Ecology 43(1), 1-11.

Hülsen, T., Barry, E.M., Lu, Y., Puyol, D. and Batstone, D.J. (2016b) Low temperature treatment of domestic wastewater by purple phototrophic bacteria: Performance, activity, and community. Water Research 100, 537-545.

749 Hülsen, T., Barry, E.M., Lu, Y., Puyol, D., Keller, J. and Batstone, D.J. (2016a)
750 Domestic wastewater treatment with purple phototrophic bacteria using a novel
751 continuous photo anaerobic membrane bioreactor. Water Research 100, 486-495.

752 Hülsen, T., Hsieh, K., Lu, Y., Tait, S. and Batstone, D.J. (2018) Simultaneous
753 treatment and single cell protein production from agri-industrial wastewaters using
754 purple phototrophic bacteria or microalgae – A comparison. Bioresource Technology
755 254, 214-223.

756 Imam, S., Noguera, D.R. and Donohue, T.J. (2013) Global insights into energetic
757 and metabolic networks in Rhodobacter sphaeroides. BMC Systems Biology 7(1),
758 89.

759 Izu, K., Nakajima, F., Yamamoto, K. and Kurisu, F. (2001) Aeration conditions
760 affecting growth of purple nonsulfur bacteria in an organic wastewater treatment
761 process. Systematic and Applied Microbiology 24(2), 294-302.

762 Janssen, M., Kuijpers, T.C., Veldhoen, B., Ternbach, M.B., Tramper, J., Mur, L.R.
763 and Wijffels, R.H. (1999) Specific growth rate of Chlamydomonas reinhardtii and
764 Chlorella sorokiniana under medium duration light/dark cycles: 13–87 s. Journal of
765 Biotechnology 70(1), 323-333.

766 Jimenez, J., Miller, M., Bott, C., Murthy, S., De Clippeleir, H. and Wett, B. (2015)
767 High-rate activated sludge system for carbon management – Evaluation of crucial
768 process mechanisms and design parameters. Water Research 87, 476-482.

769 Kiepper, B.H., Merka, W.C. and Fletcher, D.L. (2008) Proximate Composition of
770 Poultry Processing Wastewater Particulate Matter from Broiler Slaughter Plants.
771 Poultry Science 87(8), 1633-1636.

772 Kim, J.K. and Lee, B.-K. (2000) Mass production of Rhodopseudomonas palustris as
773 diet for aquaculture. Aquacultural Engineering 23(4), 281-293.

774 Kim, M.K., Choi, K.-M., Yin, C.-R., Lee, K.-Y., Im, W.-T., Lim, J.H. and Lee, S.-T.
775 (2004) Odorous swine wastewater treatment by purple non-sulfur bacteria,
776 Rhodopseudomonas palustris, isolated from eutrophicated ponds. Biotechnology
777 Letters 26(10), 819-822.

778 Kobayashi, M. and Tchan, Y.T. (1973) Treatment of industrial waste solutions and
779 production of useful by-products using a photosynthetic bacterial method. Water
780 Research 7(8), 1219-1224.

781 Kobayashi, M., Kobayashi, M. and Nakanishi, H. (1971) Construction of a purification
782 plant for polluted water using photosynthetic bacteria. J. Ferment. Technol. 49(9),
783 817-825.

784 Lo, Y.M., Cao, D., Argin-Soysal, S., Wang, J. and Hahm, T.-S. (2005) Recovery of
785 protein from poultry processing wastewater using membrane ultrafiltration.
786 Bioresource Technology 96(6), 687-698.

787 Loo, P.L., Chong, V.C., Ibrahim, S. and Sabaratnam, V. (2015) Manipulating culture
788 conditions and feed quality to increase the survival of larval marble goby Oxyeleotris
789 marmorata. North American Journal of Aquaculture 77(2), 149-159.

790 Lu, H., Han, T., Zhang, G., Ma, S., Zhang, Y., Li, B. and Cao, W. (2017) Natural
791 light-micro aerobic condition for PSB wastewater treatment: a flexible, simple, and
792 effective resource recovery wastewater treatment process. Environmental
793 Technology, 1-9.

794 Masella, A., Bartram, A., Truszkowski, J., Brown, D. and Neufeld, J. (2012)
795 PANDAseq: paired-end assembler for illumina sequences. BMC Bioinformatics
796 13(1), 31.

797 Matassa, S., Batstone, D.J., Hülsen, T., Schnoor, J. and Verstraete, W. (2015) Can
798 direct conversion of used nitrogen to new feed and protein help feed the world?
799 Environmental Science & Technology 49(9), 5247-5254.

800 McCarty, P.L., Bae, J. and Kim, J. (2011) Domestic Wastewater Treatment as a Net
801 Energy Producer–Can This be Achieved? Environmental Science & Technology
802 45(17), 7100-7106.

803 McMurdie, P.J. and Holmes, S. (2013) phyloseq: an R package for reproducible
804 interactive analysis and graphics of microbiome census data. PLOS ONE 8(4),
805 e61217.

806 Melis, A. (2009) Solar energy conversion efficiencies in photosynthesis: Minimizing
807 the chlorophyll antennae to maximize efficiency. Plant Science 177(4), 272-280.

808 Mirón, A.S., García, M.C.C., Gómez, A.C., Camacho, F.G.a., Grima, E.M. and Chisti,
809 Y. (2003) Shear stress tolerance and biochemical characterization of Phaeodactylum
810 tricornutum in quasi steady-state continuous culture in outdoor photobioreactors.
811 Biochemical Engineering Journal 16(3), 287-297.

812 Mo, W. and Zhang, Q. (2013) Energy–nutrients–water nexus: Integrated resource
813 recovery in municipal wastewater treatment plants. Journal of Environmental
814 Management 127, 255-267.

815 Murphy, T.E. and Berberoglu, H. (2014) Flux balancing of light and nutrients in a
816 biofilm photobioreactor for maximizing photosynthetic productivity. Biotechnology
817 Progress 30(2), 348-359.

818 Ogbonna, J.C., Yoshizawa, H. and Tanaka, H. (2000) Treatment of high strength
819 organic wastewater by a mixed culture of photosynthetic microorganisms. Journal of
820 Applied Phycology 12(3), 277-284.

821 Oren, A. (2014) The Prokaryotes, pp. 709-726, Springer.

822 Oswald, W.J. (1980) Algal production--problems, achievements and potential. Algae
823 biomass: production and use/[sponsored by the National Council for Research and
824 Development, Israel and the Gesellschaft fur Strahlen-und Umweltforschung (GSF),
825 Munich, Germany]; editors, Gedaliah Shelef, Carl J. Soeder.

826 Oswald, W.J. and Golueke, C.G. (1960) Advances in applied microbiology, pp. 223-
827 262, Elsevier.

828 Park, J.B.K., Craggs, R.J. and Shilton, A.N. (2011) Wastewater treatment high rate
829 algal ponds for biofuel production. Bioresource Technology 102(1), 35-42.

830 Pearson, W.R., Wood, T., Zhang, Z. and Miller, W. (1997) Comparison of DNA
831 Sequences with Protein Sequences. Genomics 46(1), 24-36.

832 Ponsano, E., Pinto, M., Garcia-Neto, M. and Lacava, P. (2004) Performance and
833 color of broilers fed diets containing Rhodocyclus gelatinosus biomass. Revista
834 Brasileira de Ciência Avícola 6, 237-242.

835 Prasertsan, P., Choorit, W. and Suwanno, S. (1993) Optimization for growth of
836 Rhodocyclus gelatinosus in seafood processing effluents. World Journal of
837 Microbiology and Biotechnology 9(5), 593-596.

838 Puyol, D.P., Batstone, D.J., Hülsen, T., Astals, S., Peces, M. and Krömer, J.O.
839 (2016) Resource recovery from wastewater by biological technologies: opportunities,
840 challenges and prospects. Frontiers in Microbiology 7, 2106.Qi, Z., Zhang, X.-H.,
841 Boon, N. and Bossier, P. (2009) Probiotics in aquaculture of China — Current state,
842 problems and prospect. Aquaculture 290(1–2), 15-21.

843 Qi, Z., Zhang, X.-H., Boon, N. and Bossier, P. (2009) Probiotics in aquaculture of
844 China — Current state, problems and prospect. Aquaculture 290(1–2), 15-21.

845 Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J.
846 and Glöckner, F.O. (2012) The SILVA ribosomal RNA gene database project:
847 improved data processing and web-based tools. Nucleic Acids Research 41(D1),
848 D590-D596.

849 Ramsay, I. and Pullammanappallil, P. (2001) Protein degradation during anaerobic
850 wastewater treatment: derivation of stoichiometry. Biodegradation 12(4), 247-256.

851 Rockström, J., Steffen, W., Noone, K., Persson, Å., Chapin III, F.S., Lambin, E.,
852 Lenton, T., Scheffer, M., Folke, C. and Schellnhuber, H.J. (2009) Planetary
853 boundaries: exploring the safe operating space for humanity. Ecology and society
854 14(2).

855 Rosenberg, E. (2014) The Prokaryotes: Other Major Lineages of Bacteria and The
856 Archaea. Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E. and Thompson, F.
857 (eds), pp. 493-495, Springer Berlin Heidelberg, Berlin, Heidelberg.

858 Rusten, B., Siljudalen, J.G., Wien, A. and Eidem, D. (1998) Biological pretreatment
859 of poultry processing wastewater. Water Science and Technology 38(4-5), 19-28.

860 Samocha, T.M., Davis, D.A., Saoud, I.P. and DeBault, K. (2004) Substitution of fish
861 meal by co-extruded soybean poultry by-product meal in practical diets for the
862 Pacific white shrimp, Litopenaeus vannamei. Aquaculture 231(1), 197-203.

863 Sasaki, K., Noparatnaraporn, N. and Nagai, S. (1991) Use of photosynthetic bacteria
864 for the production of SCP and chemicals from organic waste. In: Martin AM, ed.
865 Bioconversion of Waste Materials to Industrial Product. New York: Elsevier Applied
866 Science Publishers, 223–262.

867 Savage, N. (2011) Algae: The scum solution. Nature 474(7352), S15-S16.

868 Schlüter, M., Groeneweg, J. and Soeder, C.J. (1987) Impact of rotifer grazing on
869 population dynamics of green microalgae in high-rate ponds. Water Research
870 21(10), 1293-1297.

871 Schulz, S., Wagener, S. and Pfennig, N. (1990) Utilization of various chemotrophic
872 and phototrophic bacteria as food by the anaerobic ciliate Trimyema compressum.
873 European Journal of Protistology 26(2), 122-131.

874 Sforza, E., Simionato, D., Giacometti, G.M., Bertucco, A. and Morosinotto, T. (2012)
875 Adjusted light and dark cycles can optimize photosynthetic efficiency in algae
876 growing in photobioreactors. Plos one 7(6), e38975.

877 Shipman, R.H., Kao, I.C. and Fan, L.T. (1975) Single-cell protein production by
878 photosynthetic bacteria cultivation in agricultural by-products. Biotechnology and
879 Bioengineering 17(11), 1561-1570.

880 Simionato, D., Basso, S., Giacometti, G.M. and Morosinotto, T. (2013) Optimization
881 of light use efficiency for biofuel production in algae. Biophysical Chemistry
882 182(Supplement C), 71-78.

883 Slade, R. and Bauen, A. (2013) Micro-algae cultivation for biofuels: Cost, energy
884 balance, environmental impacts and future prospects. Biomass and Bioenergy 53,
885 29-38.

886 Spellerberg, I.F. and Fedor, P.J. (2003) A tribute to Claude Shannon (1916–2001)
887 and a plea for more rigorous use of species richness, species diversity and the
888 'Shannon–Wiener' Index. Global Ecology and Biogeography 12(3), 177-179.

889 Spolaore, P., Joannis-Cassan, C., Duran, E. and Isambert, A. (2006) Commercial
890 applications of microalgae. Journal of bioscience and bioengineering 101(2), 87-96.

891 Strong, P., Laycock, B., Mahamud, S., Jensen, P., Lant, P., Tyson, G. and Pratt, S.
892 (2016) The Opportunity for High-Performance Biomaterials from Methane.
893 Microorganisms 4(1), 11.

894 Taiganides, G. (1992) Pig waste management and recycling: the Singapore
895 experience, IDRC.

896 Tait, S., Tamis, J., Edgerton, B. and Batstone, D.J. (2009) Anaerobic digestion of
897 spent bedding from deep litter piggery housing. Bioresource Technology 100(7),
898 2210-2218.

899 Takeno, K., Sasaki, K., Watanabe, M., Kaneyasu, T. and Nishio, N. (1999) Removal
900 of phosphorus from oyster farm mud sediment using a photosynthetic bacterium,
901 Rhodobacter sphaeroides IL106. Journal of bioscience and bioengineering 88(4),
902 410-415.

903 Tchobanoglous, G., Burton, F. and Stensel, H. (2003) Wastewater Engineering
904 Treatment and Reuse, 4th Edn. Metcalf and Eddy. Inc. McGraw-Hill Company, 186.

905 Turpin, D.H. (1991) Effects of inorganic N availability on algal photosynthesis and
906 carbon metabolism. M. Journal of Phycology 27(1), 14-20.

907 van der Grinten, E., Janssen, A.P., de Mutsert, K., Barranguet, C. and Admiraal, W.
908 (2005) Temperature-and light-dependent performance of the cyanobacterium
909 Leptolyngbya foveolarum and the diatom Nitzschia perminuta in mixed biofilms.
910 Hydrobiologia 548(1), 267-278.

911 Wan, M., Liu, P., Xia, J., Rosenberg, J.N., Oyler, G.A., Betenbaugh, M.J., Nie, Z. and
912 Qiu, G. (2011) The effect of mixotrophy on microalgal growth, lipid content, and
913 expression levels of three pathway genes in Chlorella sorokiniana. Applied
914 Microbiology and Biotechnology 91(3), 835-844.

915 Wang, C., Zuo, J., Chen, X., Xing, W., Xing, L., Li, P., Lu, X. and Li, C. (2014)
916 Microbial community structures in an integrated two-phase anaerobic bioreactor fed
917 by fruit vegetable wastes and wheat straw. Journal of Environmental Sciences
918 26(12), 2484-2492.

919 Wang, W., Zhang, W., Wang, X., Lei, C., Tang, R., Zhang, F., Yang, Q. and Zhu, F.
920 (2017) Tracing heavy metals in ‘swine manure-maggot-chicken’production chain.
921 Scientific Reports 7(1), 8417.

922 Woodgate, S. and Van Der Veen, J. (2004) The role of fat processing and rendering
923 in the European Union animal production industry. Biotechnologie, agronomie,
924 société et environnement 8(4), 283-294.

925 Yurkov, V.V. and Beatty, J.T. (1998) Aerobic Anoxygenic Phototrophic Bacteria.
926 Microbiology and Molecular Biology Reviews 62(3), 695-724.

927 Zhang, P., Chen, Y. and Zhou, Q. (2009) Waste activated sludge hydrolysis and
928 short-chain fatty acids accumulation under mesophilic and thermophilic conditions:
929 Effect of pH. Water Research 43(15), 3735-3742.

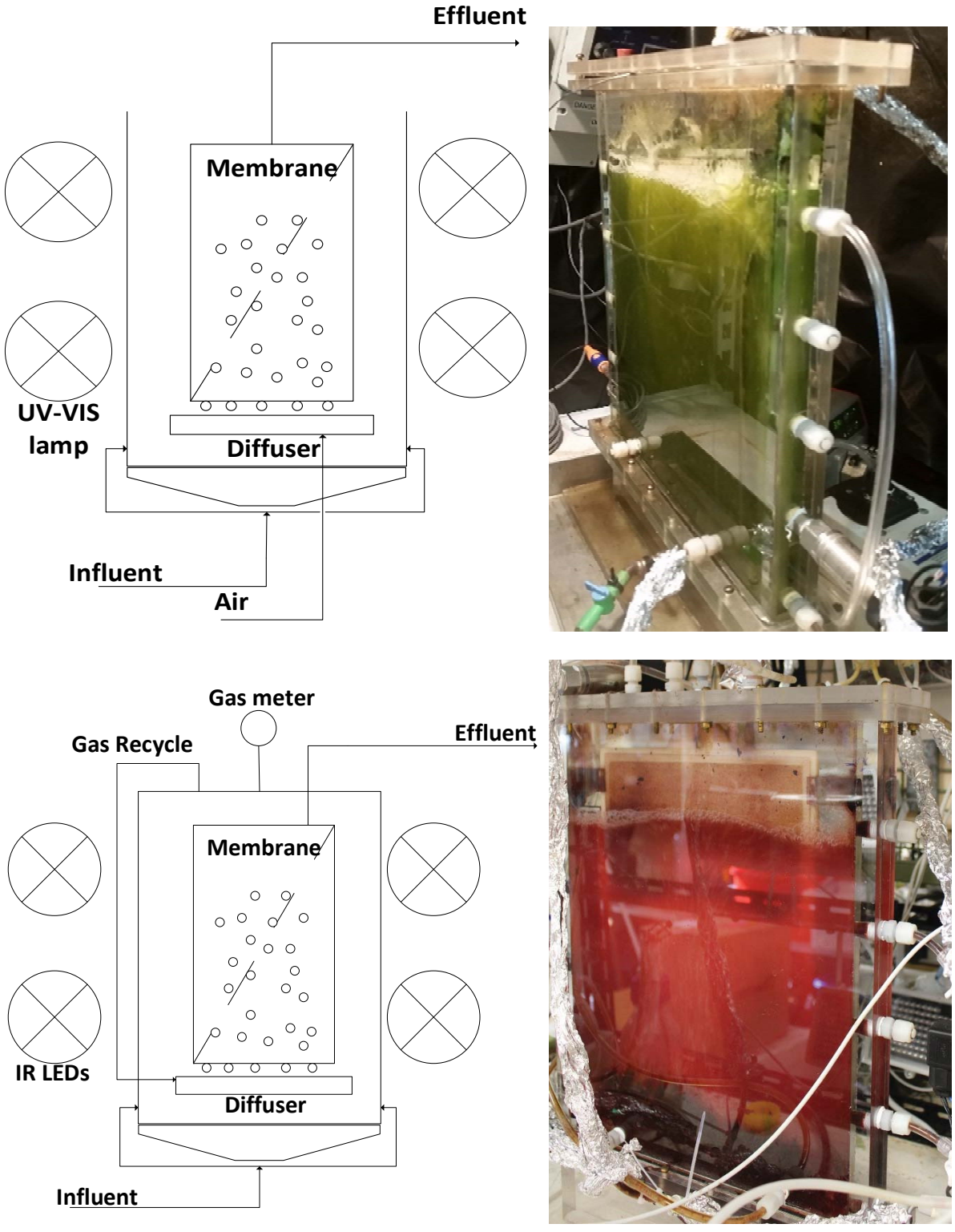


Figure 1: Schematic configuration and pictures of the WLR with algae (top) and IRR with PPB (bottom).

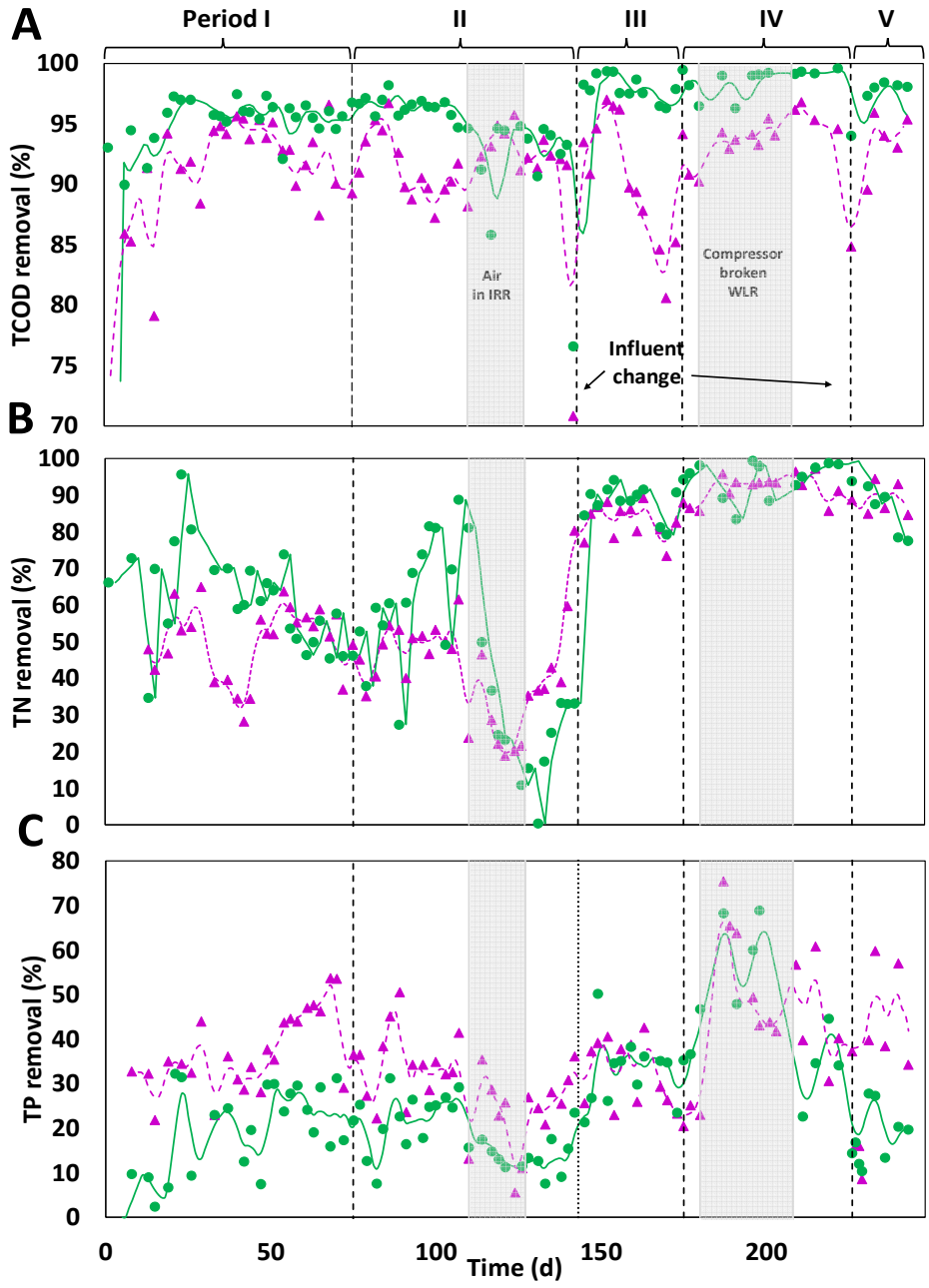


Figure 2: TCOD (A), TN (B) and TP (C) removal efficiencies of the algal WLR (●) and the PPB IRR (▲) over time. Solid (WLR) and hatched (IRR) lines represent 5 point central moving averages. The vertical dashed lines indicate a transition between operational periods and a summary of each period (D) with average TCOD, TN and TP removal efficiencies of the WLR and IRR for the various reactor operation periods (I-V).

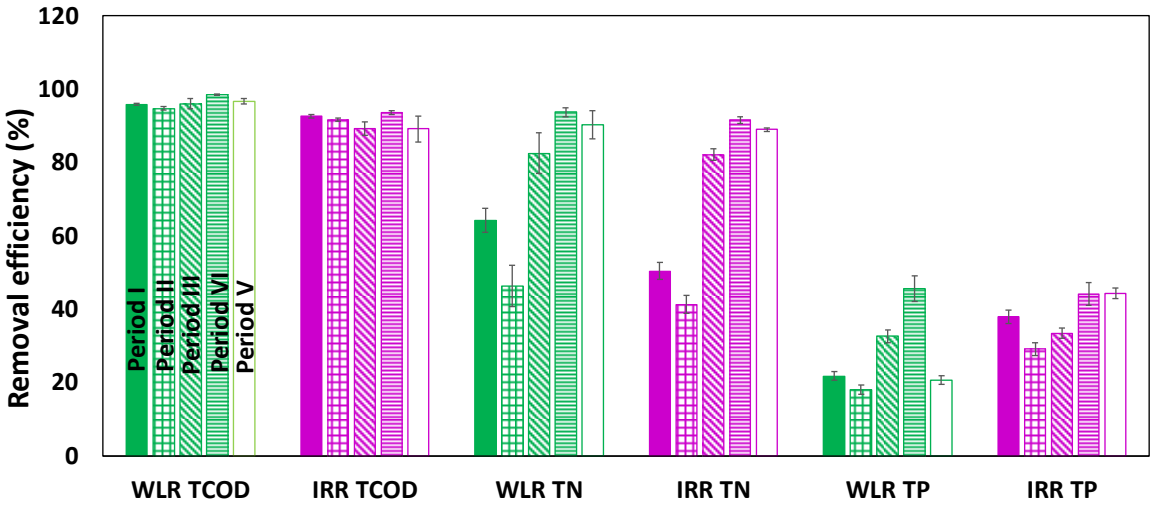


Figure 3: Average TCOD, TN and TP removal efficiencies of the WLR and IRR for the various reactor operation periods (I-V).

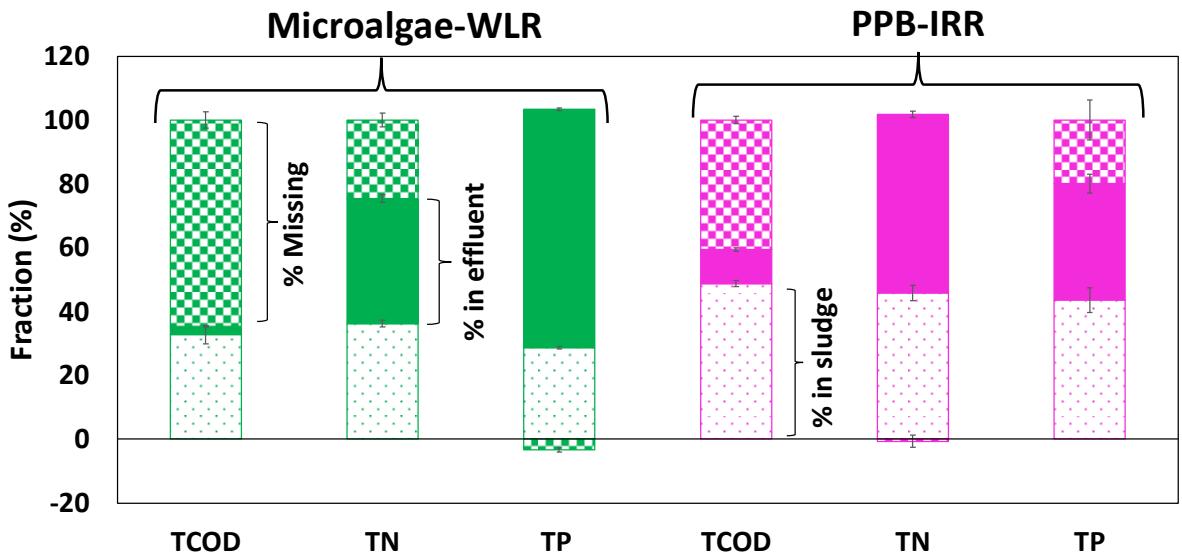


Figure 4: TCOD, TN and TP mass fractions of the WLR (green) and the IRR (pink) over 124 d, in the sludge (dotted), the effluent (solid) and missing fraction (checked). Errors bars represent 95% confidence intervals.

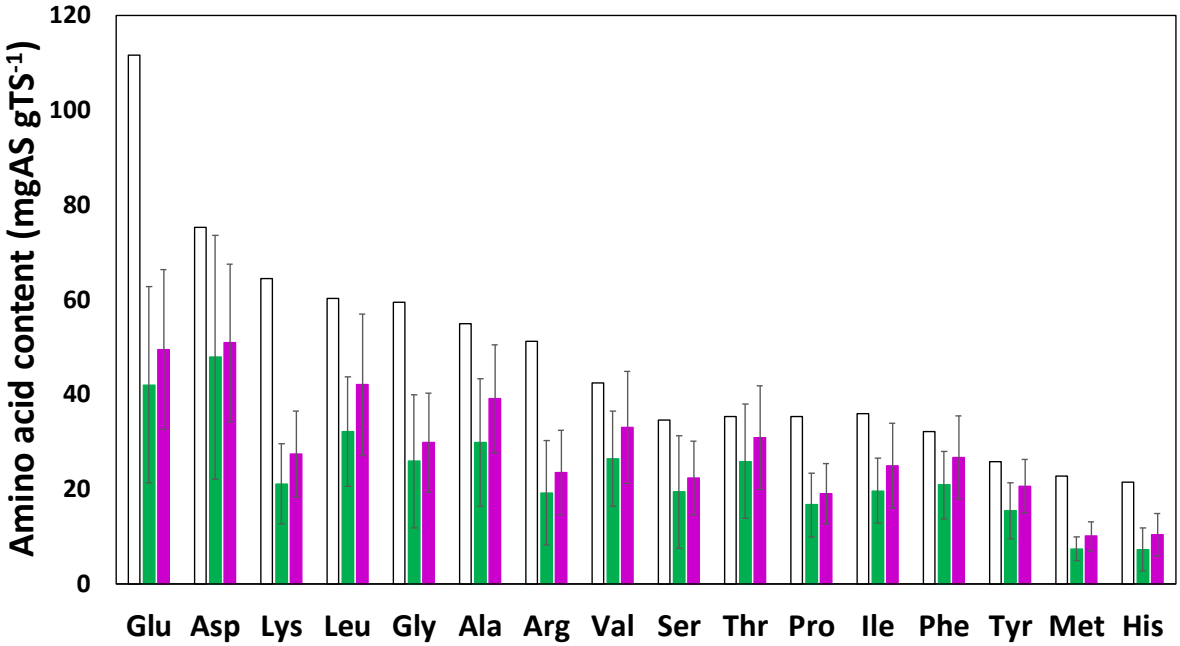


Figure 5: Reference amino acid profiles of fishmeal (white) (FAO 1970) compared with profiles of WLR biomass (green) and IRR biomass (purple) grown on the poultry processing wastewater.

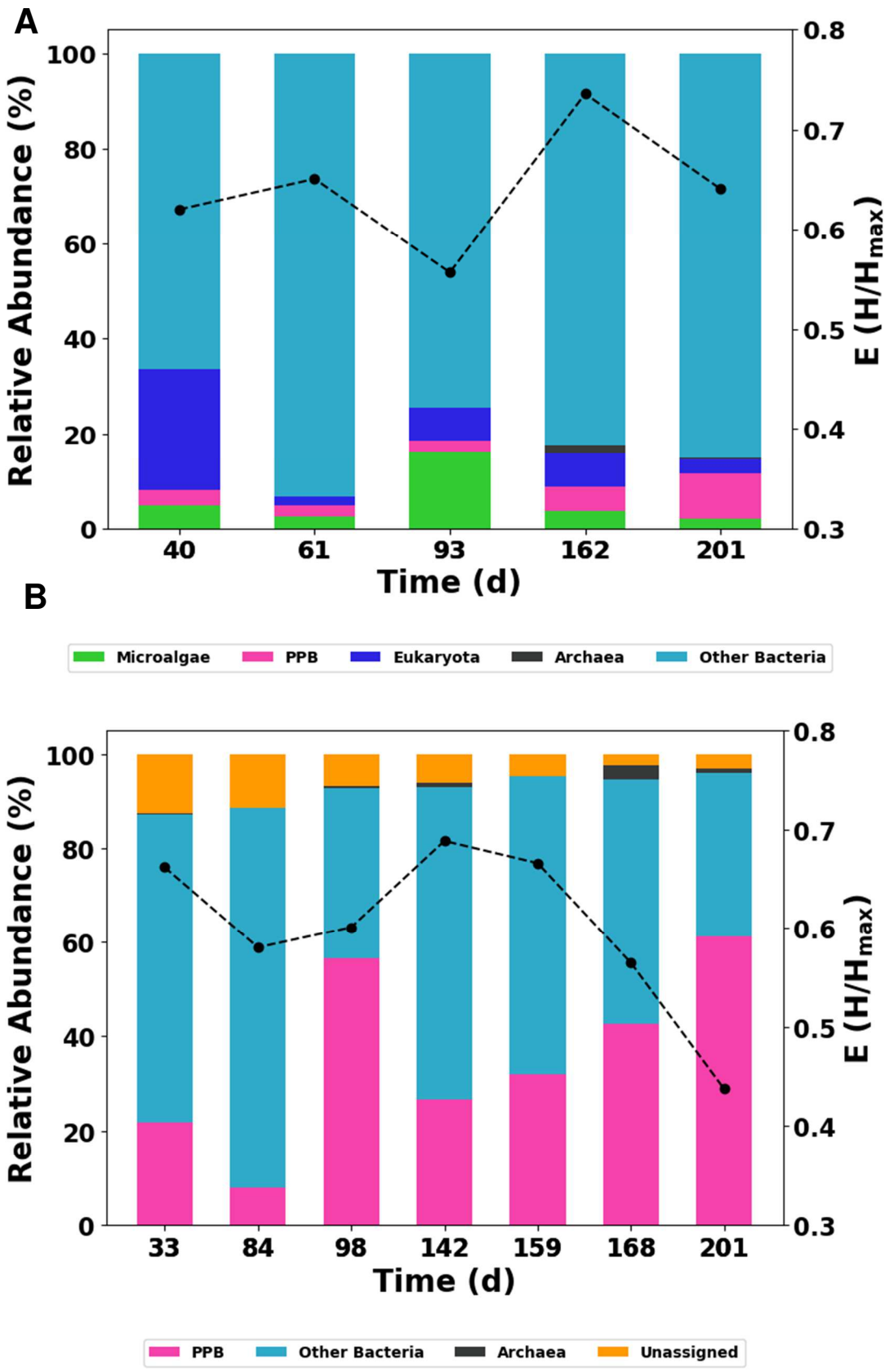
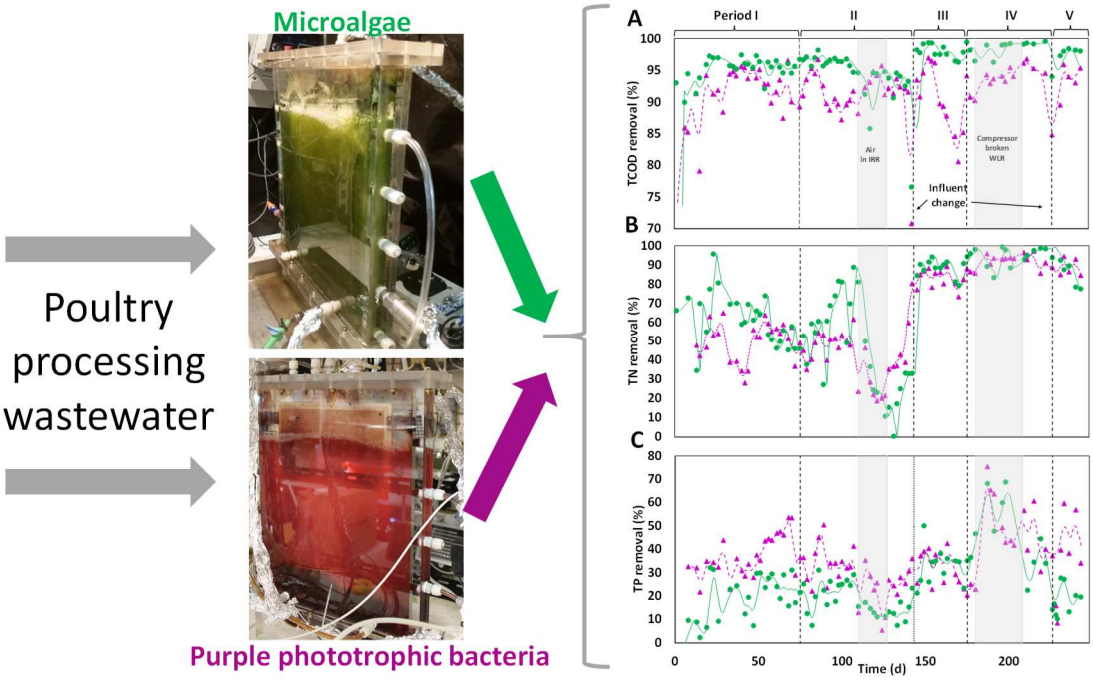


Figure 6: Relative abundance of different microbial groups and the Shannon evenness index (E) in the WLR (A) and IRR (B) treating poultry processing wastewater over time.


Microalgae
Poultry processing wastewater
Purple phototrophic bacteria
A
Period I
II
III
IV
V
TCOD removal (%)
Air in IRR
Compressor broken WLR
Influent change
B
TN removal (%)
C
TP removal (%)
Time (d)