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The effect of low temperature and low light intensity on nutrient removal from municipal wastewater by purple phototrophic bacteria (PPB)

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Abstract

There has been increased interest in alternative wastewater treatment systems to improve nutrient recovery while achieving acceptable TCOD, TN, and TP discharge limits. Purple phototrophic bacteria (PPB) have a high potential for simultaneous nutrient removal and recovery from wastewater. This study evaluated the PPB performance and its growth at different operating conditions with a focus on HRT and light optimization using a continuous-flow membrane photobioreactor (PHB). Furthermore, the effect of low temperature on PPB performance was assessed to evaluate the PPB's application in cold-climate regions. In order to evaluate PPB performance, TCOD, TN, and TP removal efficiencies and Monod kinetic parameters were analyzed at different HRTs (36, 18, and 9 h), at temperatures of 22°C and 11°C and infrared (IR) light intensities of 50, 3, and 1.4 Wm^{-2}. The results indicated that low temperature had no detrimental impact on PPB's performance. The photobioreactor (PHB) with cold-enriched PPB has a high potential to treat municipal wastewater with effluent concentrations below target limits (TCOD< $50mgL^{-1}$, TN<10 mgL^{-1}, and TP<1 mgL^{-1}). Monod kinetic parameters K_s, K, Y, and K_d were estimated at 20-29 $mgCODL^{-1}$, 1.6-1.9 $mgCOD(mgVSS.d)^{-1}$, 0.47 $mgVSS\ mgCOD^{-1}$, and 0.07-0.08 d^{-1} at temperatures of 11°C-22°C respectively. The results of the steady-state mass balances showed TCOD, TN, and TP recoveries of 80%-86%, which reflected PPB's substrate and nutrient assimilation.

Previous studies utilized high light intensities (> 50 Wm^{-2}) to provide PPB with the maximum energy required for its growth. In order to enable the PPB technology as a practical approach in municipal wastewater treatment, light intensity must be optimized. Based on the literature, there is no study on PPB performance at low light intensities using a continuous-flow membrane photobioreactor. The effect of low light intensities of 3, and 1.4 Wm^{-2} on PPB performance was addressed in this study. The results indicated that PPB at a light intensity as low as 1.4 Wm^{-2} were able to treat municipal wastewater with effluent concentrations below above-mentioned target limits. Light intensity (1-50 Wm^{-2}) had no detrimental impact on PPB performance and Monod kinetic parameters. This study showed that the optimized light intensity required for municipal wastewater treatment with PPB is significantly lower than previously indicated in the literature.

The energy consumptions attributed to PHB's illumination of 3, and 1.4 Wm^{-2} were determined to be 1.44, and 0.67 kWh/m^3 which is significantly lower than previous studies (> 24 kWh/m^3).

Keywords

Purple phototrophic bacteria; Municipal wastewater; Monod kinetic parameters; Nutrient removal and recovery; Photobioreactor; Optimization

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Co-Authorship Statement

This MESc thesis contains materials that are currently under review or in preparation to be submitted in peer reviewed journals as listed below:

Chapter 3: Low temperature nutrient removal and recovery from municipal wastewater by purple phototrophic bacteria (PPB)

Peyman Dalaei, Dang Ho, George Nakhla, Domenico Santoro

Under review in *Bioresource Technology*

The primary author of this chapter was Peyman Dalaei under the supervision of Dr. Nakhla and Dr. Santoro. The experiments were planned and conducted with guidance from Dr.Nakhla, Dr. Santoro, and Dr. Ho. The experimental analysis, data collection, and drafting the manuscript was carried out by Peyman Dalaei. Feedback on the manuscript was received from Dr. Nakhla and Dr. Santoro.

Chapter 4: The effects of low light intensity on purple phototrophic bacteria (PPB) performance for municipal wastewater treatment

Peyman Dalaei, Dang Ho, George Nakhla, Domenico Santoro

In preparation for submission to *Bioresource Technology*

The experiments was planned and conducted with guidance from Dr.Nakhla, Dr. Santoro, and Dr. Ho. The experimental analysis, data collection, and drafting the manuscript was carried out by Peyman Dalaei. Feedback on the manuscript was received from Dr. Nakhla and Dr. Santoro.

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List of Abbreviations and Symbols

BOD   biochemical oxygen demand (mg/L)

BOD5   5-day biochemical oxygen demand (mg/L)

COD   chemical oxygen demand (mg/L)

DO   dissolved oxygen (mg/L)

HRAP   high rate algal ponds

HRT   hydraulic retention time (h)

IR   infra-red

ISS   inert suspended solids (mg/L)

K   maximum specific substrate utilization rate (gCOD/gVSS-d)

K_d endogenous decay coefficicent (d^{-1})

K_s half-velocity constant (mgCOD/L)

MBR   membrane bioreactor

nbCOD   non-biodegradable COD (mg/L)

nbsCOD   non-biodegradable COD (mg/L)

nbVSS   non-biodegradable votatile suspended solids

NH_4-N   ammonium nitrogen

NO_2-N   nitirte nitrogen

NO_3-N   nitrate nitrogen

PHB   photobioractor

PPB   purple phototrophic bacteria

rbCOD   readily biodegradable COD (mg/L)

rbsCOD   readily biodegradable soluble COD (mg/L)

SCOD   soluble chemical oxygen demand (mg/L)

SRT   sludge retention time (d)

TCOD   total chemical oxygen demand (mg/L)

TKN   total Kjeldahl nitrogen (mg/L)

TN   total nitrogen (mg/L)

TP   total phosphorous (mg/L)

TS   total solids (mg/L)

TSS   total suspended solids (mg/L)

VFA   volatile fatty acids (mg/L)

VSS   volatile suspended solids (mg/L)

WWTP   wastewater treatment plant

Y   true yield coefficient (mgVSS/mgCOD)

μ specific growth rate (d^{-1})

μ_{max} maximum specific growth rate (d^{-1})

Chapter 1

1. Introduction

1.1. Rationale

A key challenge of municipal wastewater treatment is resource recovery while achieving an acceptable discharge limit. The current municipal wastewater treatment systems offer a maximum recovery of 15-20% nitrogen (Fux and Siegrist, 2004), and 20-30% phosphorous (Pitman et al., 1991; Jaffer et al., 2002). Environmental, financial costs of energy as well as the availability and costs of mineral fertilizers are the reasons behind shifting wastewater treatment from treatment towards resource recovery. In view of the paradigm shift in the wastewater industry towards resource recovery, novel technologies that recover organics, and nutrients such as algae and phototrophic bacteria are emerging. Algae and high rate algal pond (HRAP) can partition nutrients from wastewater using light. However, the application of HRAP is limited by its large footprint due to illuminated surface to volume ratios of 4-8 m^2/m^3. Wastewater treatment by algae also requires high operational costs due to long HRTs between 2-10 days (Jorquera et al., 2010). Another possibility is the nutrient recovery and removal by purple phototrophic bacteria (PPB) which uses less light per weight assimilated biomass in comparison with algae (Basak and Das., 2009). Limited research has been conducted thus far to evaluate municipal wastewater treatment by PPB (Hulsen et al., 2014; Hulsen et al., 2016). The main goal of the previous studies was to evaluate the possibility of wastewater treatment by PPB (Chitaporpan et al., 2013; Hulsen et al., 2016). High light intensities were used to provide PPB with the maximum energy required for growth in wastewater. The PPB technology was not being optimized, had high operational costs, especially for lighting, which is a major concern and is required to be optimized to enable the practical application of the PPB in wastewater treatment industry.

1.2. Objectives

The objectives of this study are: (a) the evaluation of PPB performance at room and low temperature using a continuous-flow membrane photobioreactor (PHB); (b) the effects of low light intensities of 3, and 1.4 Wm^{-2} on PPB performance in comparison with its performance at a high light intensity of 50 Wm^{-2}; (c) the effects of temperature and light intensity on PPB enrichment and its growth; (c) the assessment of the simultaneous removal of COD, TN, and TP to below target limits of 50 $mgCODL^{-1}$, 10 $mgNL^{-1}$, and 1 $mgPL^{-1}$; (d) determination of the optimum HRT at low temperature and low light intensity; (e) the evaluation of the Monod kinetic parameters at low temperature and low light intensity in comparison with the kinetics at room temperature and high light intensity respectively.

1.3. Thesis organization

Chapter 1 presents an overview of the thesis and the logic behind nutrient removal and recovery from municipal wastewater by PPB. It summarizes the relevant literature to this study as well as specific research objectives. Chapter 2 includes a comprehensive literature review on wastewater treatment by PPB at different operational conditions using batch tests and continuous-flow membrane photobioreactor. Chapter 3 is a research article entitled "Low temperature nutrient removal and recovery from municipal wastewater by purple phototrophic bacteria (PPB)". The objective of this study was: (a) to evaluate the effects of low temperature on PPB enrichment and its growth; (b) to remove COD, TN, and TP from municipal wastewater to below target limits; and (c) to determine low temperature kinetics, which is critical for successful design and operation given the low system SRT of 3 days and the inherent sensitivity of the effluent substrate concentration. Chapter 4 is a research article entitled "The effects of low light intensity on purple phototrophic bacteria (PPB) performance for municipal wastewater treatment". The objective of this study was to assess PPB performance at low light intensities (3, and 1.4 Wm^{-2}), simultaneous removal of COD, TN, and TP to below target limits, and the determination of Monod kinetic parameters at low light intensity.

1.4. References

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Chapter 2

2. Literature review

2.1. Purple phototrophic bacteria (PPB)

Purple phototrophic bacteria (PPB) are a major group of phototrophic microorganisms that are divided into two different groups: purple sulfur bacteria (PSB), and purple non-sulfur bacteria (PNSB). The physiology of PSB is related to sulfide, hence high growth rate of PSB is observed under phototrophic conditions where sulfide is present (Pfennig, 1967). PNSB are a physiologically versatile group of purple bacteria which can grow substantially in anoxic phototrophic conditions with either organic carbon or carbon dioxide (CO_2). PPB require anoxic conditions for phototrophic growth because of the repression of pigment synthesis by oxygen (Cohn-Bazine et al., 1957). PPB generate energy from light in the form of adenosine triphosphate (ATP), rather than from other chemicals (Basak and Das, 2007). PPB's ability to generate energy is due to the presence of photosynthetic pigments, bacteriochlorophyll and carotenoid. PPB contain bacteriochlorophyll a (BChl a) with a maximum absorption at the spectrum of 800 nm, and BChl b with the highest absorption at 850 nm, revealing a high growth rate of PPB near the infrared (IR) region. PPB's capability to utilize infrared (IR) light is an operational advantage because IR illumination from light emitting diodes (LEDs) saves up to 70% of the power consumption compared to white lights which are required for algae growth (Bertling et al., 2006). The colors of the dense PPB culture include purple, purple-red, purple-violet, red, orange, brown, and yellow due to the presence of BChl a and BChl b and carotenoid pigments.

 PPB's structure includes light harvesting complexes (LH_1-LH_2), reaction center, cytochrome bc_1 and ATP synthase which are shown below in Figure 1 (Feniouk and Junge, 2008). LH_1 complexes have a constant characteristic which is directly associated with the reaction center, however, the amount of LH_2 and its properties vary according to the light intensity and growth conditions (Kuhlbrandt, 1995). In response to a short flash of light, light harvesting complexes harvest the photons and the PPB's reaction center generates quinol (QH_2). The subsequent oxidation of quinol by cytochrome bc_1, causes the proton pumping from cytochrome bc_1 complex to periplasm. The resultant charge separation between cytoplasm and periplasm derives proton through ATP *synthase* to generate ATP energy (Figure 2.1).

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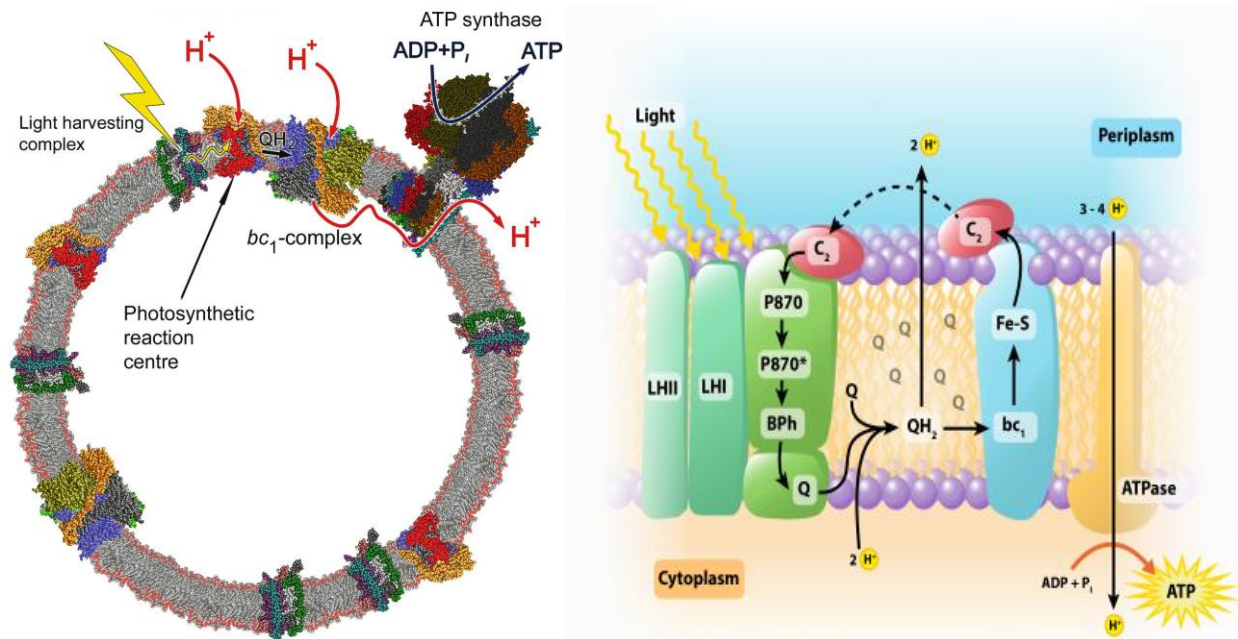
Figure 2.1. Purple phototrophic bacteria structure (Feniouk and Junge, 2008)

The PPB biomass is rich in lipids, single cell protein (SCP), biopolymers, fatty acids and phototrophic pigments such as carotenoid and bacteriochlorophyll (Sasaki et al., 2005). Carotenoid pigments are widely used in the food processing industry as colorants, vitamin precursors, and as antioxidants (Mortensen, 2006). PPB are also used as a diet ingredient with a high content of protein, vitamins, and as a disease-preventing agent in the aquaculture production (Banerjee et al., 2000). Because of PPB's ability to remove pollutants and organic matters, PPB are also employed as bioremediation agents (Lu et al., 2013).

2.2. Wastewater treatment by purple phototrophic bacteria

PPB are important microorganisms because of (1) the removal of toxic substances, H_2S, and the contribution of the organic matters to anoxic environments in their roles as autotrophs; (2) the removal of organic compounds, and primarily non-fermentable organic compounds by their photoheterotrophic capacities. Readily biodegradable organic compounds such as malate and pyruvate are the preferred substrates, however, short-chain fatty acids and even carbohydrates are utilized by PPB (Sojka, 1978). PPB have a high potential in wastewater treatment attributed to the organic carbon and CO_2 assimilation (Azad et al., 2001), the removal of ammonia as a source of

nitrogen via assimilation (Takabatake et al., 2000), as well as phosphorous uptake by polyphosphate (polyP) formation (Hiraishi et al., 1991).

McKinlay and Harwood, (2010) indicated that *R.palustris* (PPB strain) biomass formula is $CH_{1.8}O_{0.38}N_{0.18}$ and COD to VSS ratio was determined to be 1.78. In order to achieve effluent nitrogen and phosphorous concentrations below 10 mgL^{-1} and 1 mgL^{-1} respectively for the municipal wastewater treatment, the COD: N, COD:P, and N:P ratios are crucial (Hulsen et al., 2014). Hulsen et al. (2016) indicated that PPB removed organics and nutrients at an average TCOD:TN:TP ratio of 100:6:1. This ratio is higher than the TCOD:TN ratio of 100:2.5 achieved by Suhaimi et al. (1987) and higher compared to the TCOD:TP ratio of 100:0.63 reported by Hirashi et al. (1991).

Hydrogenase and *nitrogenase* present in the PPB membrane are active. *Hydrogenase* plays a major role in the fermentative hydrogen and organic acid production in the dark (Woodward et al., 2000). Although *hydrogenases* are present in PPB, phototrophic hydrogen production by the PPB is mostly mediated by *nitrogenase*. The high ammonia concentration is the reason behind the inhibition of PPB's *nitrogenase* enzyme resulting in very low hydrogen production, however, hydrogen production increased using N_2 as a nitrogen source (Kim et al., 2012a). The optimum light intensities for PPB biomass growth and photoproduction of hydrogen were achieved at 4-6 klux (32-47 Wm^{-2}) and 6-10 klux (47-79 Wm^{-2}) respectively. Light intensities higher than the optimum above-mentioned values do not cause inhibition for neither hydrogen production nor cell growth (Sasikala et al., 1991b). However, Zhou et al. (2014) indicated that the optimum light intensity to achieve the highest biomass production and COD removal from synthetic wastewater was 2 klux (16 Wm^{-2}) in batch tests using a PPB strain (*Rhodopseudomonas*). The wastewater had a TCOD, NH$_4$-N and TP of 6000, 400, and 90 mgL^{-1} respectively. The highest carotenoid content of the PPB was achieved at 8 klux (72 Wm^{-2}) with a COD removal efficiency of 87%. The highest ATP production was achieved at 2 klux (16 Wm^{-2}), however, carotenoid content of the PPB was the lowest at this light intensity.

A key advantage of using PPB in wastewater treatment is the production of high value protein. Hulsen et al, (2017) demonstrated that the crude protein, fat, and carbohydrate contents of PPB biomass are 65%, 33%, and 2% respectively. The average TCOD to VSS ratio was determined to be 1.67 (0.6 gVSS as PPB biomass for 1 gCOD$_{removed}$).

The operating conditions and main findings from the previous studies are summarized below, in Table 2.1.

Table 2.1. Summary of operating conditions and findings from the previous studies

Study	Wastewater	Experimental setup	IR intensity (Wm^{-2})	PPB culture	HRT (d)	Findings
Chitaporpan et al., 2013	Synthetic WW (PPB enrichment) Food processing WW	Membrane photobioreactor	270	Mixed	10	1. Average COD removal of 58% 2. High light intensity to prove the PPB potential for wastewater treatment
Prasertsan et al., 1993	Tuna condensate	Batch tests	8-24-40	RG[a]		1. Optimal PPB growth at pH of 7 and IR intensity of 24 Wm^{-2} 2. Average COD removal of 78% 3. Lower COD removal efficiency at low IR light intensity
Kaewsuk et al., 2010	Dairy wastewater	Continuous photobioreactor	1300	Mixed	10	1. Average COD removal of 93% 2. PPB Monod kinetic parameters determination of K_s=174 mgCODL^{-1}, K=7.42 mgCOD (mgVSSd)$^{-1}$, K_d=0.14 (d^{-1}), and μ=1.69 (d^{-1})

Reference	Wastewater	Reactor				Findings
Kim et al., 2004	Swine wastewater	Batch tests	8-16-24-30-40	RP[b]		1.Optimal PPB growth at IR intensity of 30 Wm^{-2} and a temperature of 30°C 2.The COD and phosphate removal efficiencies of 50% and 58% 3.Low substrate removal at $4Wm^{-2}$ (Organic acid removal efficiency of 20%)
Chiemchaisri et al., 2008	Noodle wastewater	Continuous photobioreactor	-	Mixed	6-10	1.High COD removal efficiency of 90%
Hulsen et al., 2014	Municipal wastewater	Batch tests	50	Mixed		1.Simultanuous removal of SCOD, NH_4-N, and PO_4-P 2.High ammonia, and phosphate removal efficiencies (>90%) with the addition of acetate
Hulsen et al., 2016	Municipal wastewater	Continuous membrane photobioreactor	50	Mixed	<1	1.Effluent TCOD, TN, and TP concentration below 50 mgL^{-1}, 10 mgL^{-1}, and 1 mgL^{-1} with the addition of an external carbon source 2.High nutrient mass closure (>90%)

						3.PPB COD: N: P uptake ratio of 100: 6: 1
Hulsen et al., 2016	Municipal wastewater	Continuous membrane photobioreactor	50	Mixed	<1	1.Effluent TCOD, TN, and TP concentration below 100 mgL^{-1}, 10 mgL^{-1}, and 1 mgL^{-1} with the addition of an external carbon source at temperature as low as 10°C
Puyol et al., 2017	Municipal wastewater	Batch tests	50	Mixed		1. PPB Monod kinetic parameters determination of K_s=20 mgCODL^{-1}, K=2.4 mgCOD (mgVSSd)$^{-1}$, K_d=0.09 (d^{-1}), and μ=1.54 (d^{-1})

[a] *Rubrivivax gelatinosus*, [b] *Rhodopseudomonas palustris*

Previous studies mainly focused on the enrichment of a single strain of PPB from industrial wastewater utilizing batch cultivation methods (Prasertsan et al., 1993). They mostly focused on the removal of COD from wastewater. It is important to note that PPB can remove COD, nitrogen (N), and phosphorous (P) simultaneously with a specific ratio. Stoichiometry of the COD, N, and P removal by PPB is an important factor which must be taken in to account.

Prasertsan et al. (1997) conducted a series of batch tests using a PPB strain (*Rhodocyclus gelatinosus*) to treat tuna condensate (the effluent after pre-cooking tuna) at different pH levels, and light intensities to evaluate the optimized pH and light intensity based on COD removal, PPB biomass growth, bacteriochlorophyll and carotenoid pigment production. The optimized pH and light intensity were determined to be 7, and 24 Wm^{-2}.

An 8L anaerobic membrane photobioreactor was used by Chitapornpan et al. (2013) to grow a mixed culture of PPB using acetate-based enrichment media at a hydraulic retention time (HRT) of 10 days and at temperatures between 28°C and 39°C. The observed IR intensity at the front surface of the photobioreactor was 270 Wm^{-2}. The food processing wastewater had an influent COD, BOD, and TKN concentration of 3171±1692, 2046±1265, and 17±8 mgL^{-1} respectively. The average COD, and BOD removal efficiencies were determined to be 58% and 51% respectively. The energy consumption attributed to lighting the photobioreactor at 270 Wm^{-2} and a surface area of 0.04 m^{-2} was determined to be 32 kWh/m^3.

Hulsen et al. (2014) showed that PPB can be enriched in municipal wastewater alone by using a series of the batch tests under anaerobic conditions with infrared (IR) light at 30±1°C. The enriched PPB simultaneously removed the COD, NH_4-N, and PO_4-P with removal efficiencies of 63%, 99%, and 88% respectively from the primary effluent municipal wastewater. Acetate was used as an external carbon source to achieve the maximal nitrogen and phosphorous removal according the SCOD/NH_4-N/PO_4-P ratio of 100/8.6/1.4.

A continuous-flow membrane photobioreactor with ethanol supplementation at a dose of 300 $mgCODL^{-1}$ was employed by Hulsen et al. (2016) to remove organics and nutrients from the municipal wastewater to below discharge limits (< 50 $mgCODL^{-1}$, 10 $mgNL^{-1}$, 1 $mgPL^{-1}$). The photobioreactor (PHB) was operated at high IR light intensity of 50 Wm^{-2} to prove the application of the PPB to treat the municipal wastewater using PHB at HRT of 8-24 h. Steady state mass balances indicated the average recoveries of TCOD, TN, and TP of 78%, 91%, and 92% respectively. The effluent TCOD, TN, and TP concentration were 30±10 mgL^{-1}, 4.3±1.2 mgL^{-1}, and 0.45±0.3 mgL^{-1} respectively, corresponding to the TCOD, TN, and TP removal efficiencies of 96.9%±0.9%, 92%±2.3%, and 94.6%±3.1%. Mixing energy for membrane scouring and lighting are the main inputs for anaerobic membrane photobioreactor using PPB. PHB's energy consumption attributed to the light intensity of 50 Wm^{-2} was determined to be 24 kWh/m^3 (Hulsen et al.,2016). Hulsen et al. (2017) estimated the energy required for the PHB' mixing to 0.15 kWh/m^3. Therefore, the high energy required for the PHB's lighting is the main concern for the PPB's technology, thus necessitating optimization.

A series of anaerobic batch tests at 30°C using 160 mL flasks were used by Huslen et al. (2017) to evaluate PPB performance in the treatment of filtered red meat processing wastewater. The NH_4-

N and PO$_4$-P removal efficiencies were 64% and 73% respectively. The results from this study indicated that the ratio of SCOD/NH$_4$-N/PO$_4$-P removed by PPB was 100/4.3/1.7. The overall PPB yield on the filtered red meat processing wastewater was 0.9±0.1 gCOD$_{produced}$ (gCOD$_{removed}$)$^{-1}$, which demonstrates the potential of high recovery in wastewater treatment by PPB. The mass balance closure showed that the removal of nitrogen was non-destructive with a mass closure of 100% (Hulsen et al., 2017).

2.3. Biological methane potential from PPB biomass

The mesophilic (37°C) anaerobic digestion of PPB biomass from the filtered red meat processing wastewater indicated that the cumulative methane production at standard temperature and pressure (STP) was 381 mLCH$_4$ / gVS$_{added}$ after 42 d which is depicted in Figure 2.2 (Hulsen et al., 2017). A significant conclusion from the BMP test results showed that the total degradable fraction (f$_d$) was fitted at approximately 53%, which indicates that a considerable portion of the sludge would still require disposal.
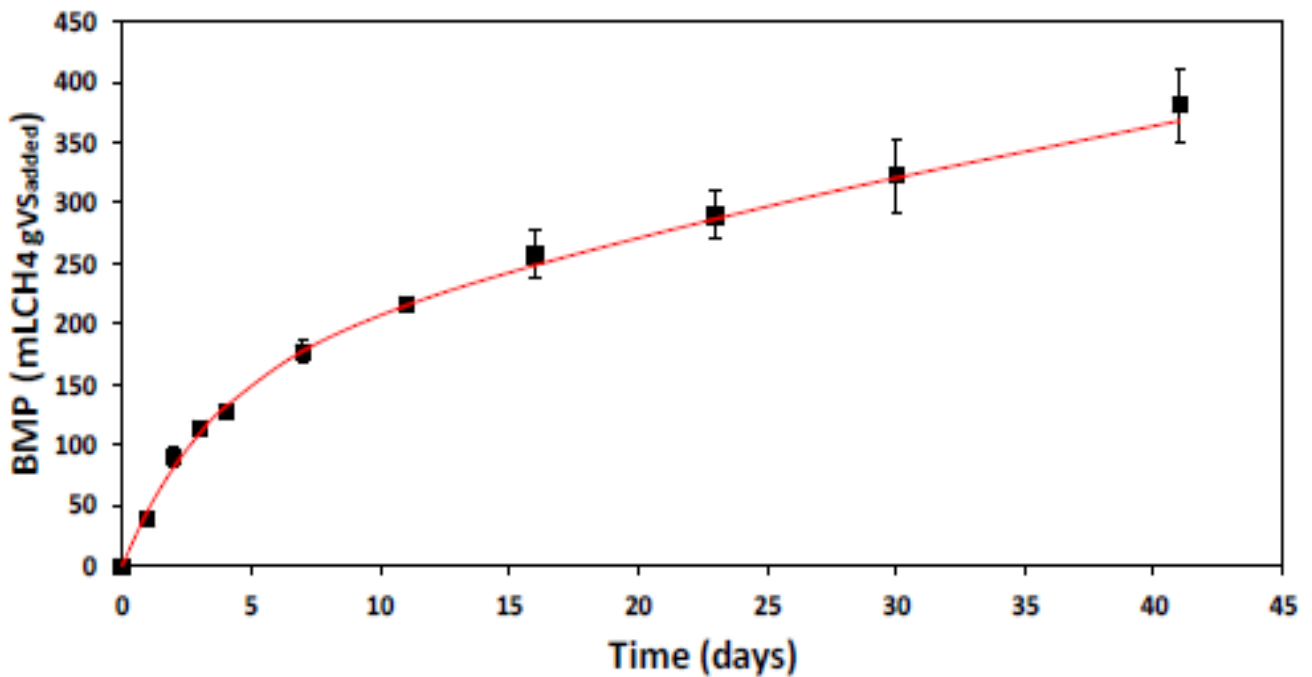
Figure 2.2. Cumulative methane production from the anaerobic digestion of PPB biomass grown in the red meat processing wastewater (Hulsen et al., 2017)

The PPB anaerobic digestion's drawback is the release of nutrients; 27 mgNH4-N gVSadded[-1] and 5.5 mgPO4-P gVSadded[-1], corresponding to 26% and 43% of nitrogen and phosphorous in the initial PPB biomass respectively (Table 2.2). However, the released COD: N: P ratio of 100: 2.6: 0.5 demonstrated the high potential of the PPB fermentate for use as an internal COD source rather than ethanol as an external carbon source.

Table 2.2. COD, TKN, and TP content of the PPB biomass and anaerobic release after 42 days

PPB biomass (t=0)		
mgTKN gVS^{-1}	mgTP gVS^{-1}	mgCOD gVS^{-1}
103.3	13	1700
Released (t=42 d)		
26.5	5.5	1
Released (%)		
26	43	59

2.4. Wastewater treatment with PPB at low temperature

Cold-active purple bacteria can grow in permanently cold environments (Burke and Burton, 1988; Madigan, 1998). A study by Karr et al. (2003) demonstrated that purple bacteria inhabited a lake in McMurdo Dry Valleys of Antarctica, which is permanently frozen with ice covers of 4-7 m. Purple bacteria were isolated from samples of microbial mats that grew along the edge of lakes and from water under the ice. *Rhodoferax antarcticus* was detected as a cold-active purple bacterium (optimal growth at 18°C and no growth occurs above 24°C), which was the first anoxygenic phototrophic bacteria that can grow at 0°C (Madigan et al., 2000). According to the literature, only one study was carried out to evaluate the wastewater treatment by PPB at low temperature. Hulsen et al. 2016 evaluated the application of phototrophic purple bacteria (PPB) for municipal wastewater treatment at low temperature (10 ± 1°C). The results indicated that simultaneous TCOD, TN, and TP removal to below discharge limits (TCOD < 100 mgL^{-1}, TN <

10 mgL^{-1}, and TP < 1 mgL^{-1}) was achieved at the HRT and SRT of 22 hours and 3 days respectively.

2.5. Monod kinetic parameters

Table 2.3 demonstrates the Monod kinetic parameters of the PPB from different studies using various kinds of substrates. A series of batch tests were performed by Puyol et al. (2017) to evaluate the Monod kinetic parameters using PPB inoculum from the photobioreactor which was operated by Hulsen et al. (2016). PPB grow rapidly in municipal wastewater under anaerobic conditions (K = 2.4 mgCOD mgVSS^{-1} d^{-1}, K$_s$= 20 mgCODL^{-1}). The hydrolysis was relatively slow (0.1 d^{-1}), which showed that the particulate substrates were not degraded at short HRTs. However, the decay rate was relatively high (K$_d$ = 0.09 d^{-1}) which was comparable to the activated sludge.

Table 2.3. The summary of Monod parameters using various kinds of substrate

Substrate	Bacteria	K$_s$ (mgCOD/ L)	K (mgCOD/ mgVSSd)	K$_d$ (d^{-1})	Y (mgVSS/ mgCOD)	μ$_m$ (d^{-1})	References
Dairy wastewater	Mixed PPB	174	7.42	0.1383	0.2281	1.69	Kaewsuk et al., (2010)
Coconut cream	Rhodobacter sphaeroides	8000		0.0008	0.1383	0.32	Kantawanichkul, (1990)
Domestic wastewater	Mixed PPB				0.36	0.008	Somiya et al., (1988)
Acetate	Rhodobacter sphaeroides	74.5[a]			0.86[b]		Nakajima, (1996)
Acetate	Rhodobacter sphaeroides			0.014			Nakajima et al., (1997)
Acetate	Mixed PPB	20	2.4	0.09	0.67	1.54	Puyol et al., (2017)

[a] Half velocity coefficient for DOC (mgDOC/L)

[b] Growth yield on carbon (g-C/g-DOC)

2.6. The effect of light intensity on PPB performance

Holt and Marr, (1965) indicated that *Rhodospirilllum rubrum* grown at low infrared (IR) light intensity contained more bacteriochlorophyll pigments than PPB grown at high light intensity. PPB is able to adapt its structure to low light intensity by an increase in the bacteriochlorophyll content. Cohen-Bazire et al. (1957) discovered that the rate of bacteriochlorophyll synthesis is inversely linked to the light intensity in *Rhodospirillum rubrum* and *Rhodopseudomonas spherioides* (PPB strains). Just as the illumination intensity is suddenly changed, the pigments adjust their populations by transient stimulation or by suppression. A decrease in light intensity causes an acceleration in the rate of bacteriochlorophyll pigment synthesis. However, an increase in the light intensity causes a suppression in pigment synthesis. The optical density of PPB grown at low light intensity showed higher values at 590, 800, and 850 nm compared to the PPB grown at high light intensity which is depicted in Figure 2.3. The peaks at the spectrum of 590, 800, and 850 nm is due to the presence of the bacteriochlorophyll pigments in PPB.
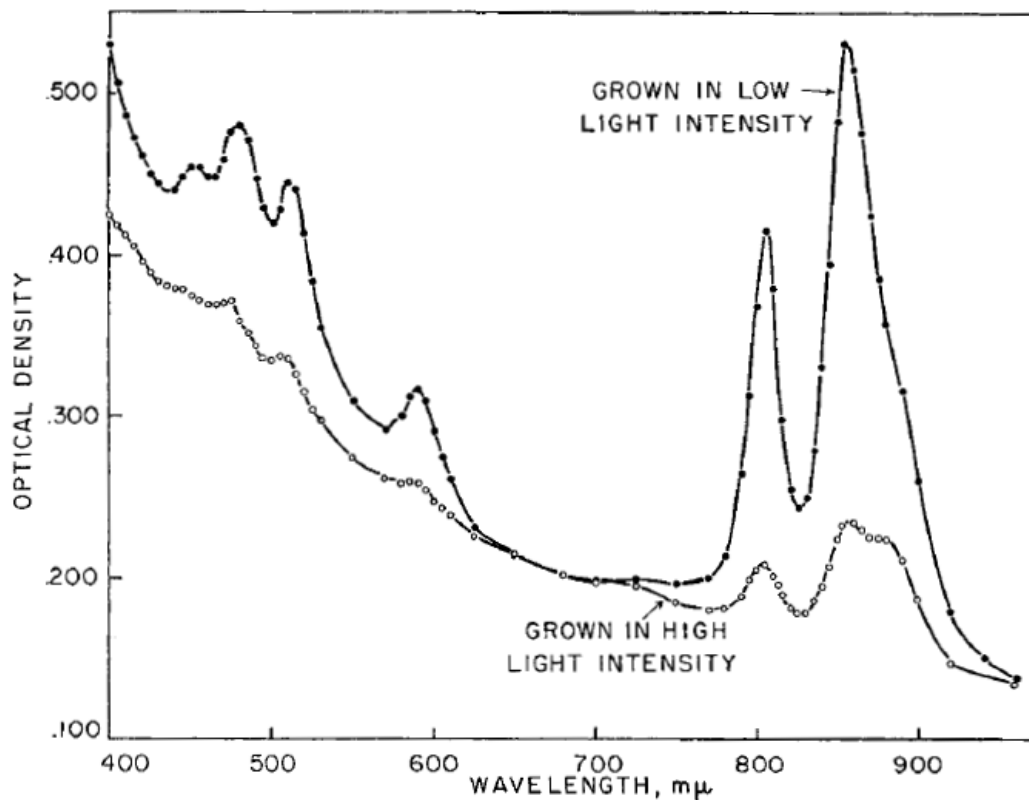
Figure 2.3. Wavelength scan of *Rhodopseudomonas speriodes* at high and low light intensities (Cohen-Bazire et al., 1957*)*

A long-term laboratory scale photobioreactor was operated by Hulsen et al. (2016) at IR light intensity of 50 Wm^{-2} to prove the PPB's ability to treat municipal wastewater. It was suggested that the photobioreactor's operation at IR light intensity of 20 Wm^{-2} may be possible which might considerably reduce the PPB operational cost (Hulsen et al., 2017). Light intensity is an important factor in the PPB growth since PPB convert light energy into chemical energy (ATP) through ATP synthase. PPB growth rate increased with an increase in the light intensity from 16 to 32 Wm^{-2}; however, further increases in the light intensity, exceeding 40 Wm^{-2}, resulted in a significant decrease in the PPB growth (Nath and Das, 2009; Shi and Yu, 2005).

A series of batch tests were conducted by Kim et al. (2004) to evaluate the removal of organic acids, COD, and phosphate from swine wastewater by PPB. The organic acids included acetic acid, isobutyric acid, propionic acid, butyric acid, valeric acid, isovaleric acid, and caproic acid. These batch tests were carried out at different operating conditions to achieve the optimum temperature, pH, and light intensity. The initial concentrations of organic acids, COD and phosphate were 169 mgL^{-1}, 18700 mgL^{-1} and 180 mgL^{-1} respectively. All experiments were performed using 150 mL serum bottles. Optimum operating conditions for the removal of organic acids by a PPB strain were determined at pH 7 and at 30°C, with a light intensity of 4 klux (32 Wm^{-2}). The removal of COD from the wastewater increased with an increase in light intensity below a specific threshold (32 Wm^{-2}). However, the COD removal efficiency dropped significantly at higher light intensities due to the light inhibition of PPB (Qu et. al., 2011; Zhang et. al., 2014). The maximum COD and PO_4-P removal efficiencies were 50% and 58% respectively when experiments were conducted under a light intensity of 32 Wm^{-2}.

Prasetsan et al. (1993) evaluated the effect of light intensities of 8, 24, and 40 Wm^{-2} on bacteriochlorophyll pigment production and COD removal from tuna condensate wastewater (diluted with shrimp-blanching water) using the *Rhodocyclus gelatinosus* PPB strain at 30°C. The amount of bacteriochlorophyll pigments of this PPB strain was maximum at 8 Wm^{-2}, however, the maximum COD removal (86%) was achieved at a light intensity of 24 Wm^{-2}.

Zhou et al. (2014) conducted a series of batch tests to determine the optimum light intensity for bacteriochlorophyll and carotenoid pigments production, PPB biomass growth, ATP production and COD removal from synthetic wastewater using the *Rhodopseudomonas* PPB strain. The initial COD, NH_4-N, and TP concentrations were respectively 6000, 400, and 90 mgL^{-1} respectively. The

batch tests were carried through using different light intensities of 500, 1000, 2000, 4000, and 8000 lux which are equivalent to 4, 8, 16, 32, and 64 Wm^{-2}.

PPB biomass production improved at light intensities of 8, 16, and 32 Wm^{-2} compared to the lowest and highest light intensities of 4 and 64 Wm^{-2} (Zhou et al., 2014). The optimum light intensity for PPB biomass was reached at 16 Wm^{-2}. PPB biomass production at 64 Wm^{-2} was lower than PPB biomass production at 4 Wm^{-2}, which means that higher light intensity inhibited PPB growth. The increase in PPB biomass production with an augmentation in light intensity from 4 to 16 Wm^{-2} is rational because a higher light intensity provided the PPB with more energy for growth; however, there was a specific light threshold at which PPB ceased to grow due to the light-inhibition (Cheirsilp and Torpee, 2012; Sorokin and Krauss, 1958). A similar phenomenon was reported for microalgae growth (Ogbonna and Tanaka, 2000; Munoz and Guieysse, 2006). It was noted that the microalgae's biomass increased continuously up to 10 klux (80 Wm^{-2}), however, there was a light inhibition for intensities higher than 10 klux, which brought the microalgae's growth to a standstill (Cheirsilp and Torpee, 2012).

The light conversion efficiency is defined as PPB biomass produced / light intensity used. The maximum and minimum light conversion efficiencies were achieved at 0.5 klux (4 Wm^{-2}) and 8 klux (64 Wm^{-2}) respectively.

The effect of different light intensities on bacteriochlorophyll and carotenoid pigments production was reported by Zhou et al. (2014). The production of carotenoid pigments was highest at 8 klux (64 Wm^{-2}) and lowest at 2klux (8 Wm^{-2}). The bacteriochlorophyll pigment production trend was significantly different with carotenoid production. The amount of bacteriochlorophyll pigments was highest at 1 klux (8 Wm^{-2}), and lowest at 8 klux (64 Wm^{-2}). The quantity of carotenoid and bacteriochlorophyll produced by PPB directly determines the energy captured by PPB and affects its growth. Bacteriochlorophyll and carotenoid pigments both exist in PPB cells and bond with protein components of the photosynthetic system (Kuo et al., 2012). Carotenoid played an important role when light intensity was excessive. Carotenoid pigments showed photo-protective abilities based on their specific structural and chemical characteristics (Connelly et al., 1997). This signifies that carotenoid pigments absorb greater light when the light intensity is near PPB's light threshold in order to protect the PPB from light inhibition.

The intracellular energy of PPB is produced in the form of adenosine triphosphate (ATP). ATP plays a significant role in various PPB activities. ATP participates in the synthesis of all intracellular substances (carbohydrate, protein, lipid, and nucleic acid) (Rasmuson et al., 2008). The Calvin cycle is the main synthesis pathway in PPB intracellular macromolecules. The Calvin cycle and synthesis process of intracellular protein continuously consume ATP, and hence require a large amount of ATP (Horton et al., 2003; Harun et al., 2002). ATP is crucial in the wastewater treatment by PPB, the removal of substrates and nutrients as well as the synthesis of PPB. As such, the amount of ATP directly affects COD removal and biomass growth. ATP production showed higher value at light intensities of 1-4 klux (8-32 Wm^{-2}) compared to a low light intensity of 500 lux (4 Wm^{-2}) (Zhou et al., 2014). The optimum light intensity for ATP production was 2 klux (16 Wm^{-2}) and its lowest ATP production was at the light intensity of 8 klux (64 Wm^{-2}).

The COD removal efficiency improved from 85% to 95% with an increase in light intensity from 1 klux to 4 klux (8-32 Wm^{-2}). The optimal light intensity for COD removal was 2 klux (16 Wm^{-2}) with the COD removal efficiency of 95% (Zhou et al., 2014). COD removal from synthetic wastewater increased with higher light intensities below the threshold of 5 klux (40 Wm^{-2}). However, COD removal efficiency decreased at higher light intensities of above 5klux (40 Wm^{-2}) due to PPB's light inhibition (Zhang et al., 2014; Qu et al., 2011).

2.7. PPB performance with light-dark cycles

Light-dark cycles affect the light energy available for PPB growth. Since bacteriochlorophyll and carotenoid pigments are major light-harvesters within PPB and have a significant effect on the quantity of ATP (energy) produced (Smith et al., 2014), the light-dark cycles will certainly impact their behaviours. The effect of light-dark cycles on COD removal from synthetic wastewater with the TCOD, NH_4-N and TP concentrations of 6000, 400, and 90 mgL^{-1}, PPB biomass and bacteriochlorophyll and carotenoid pigments production was evaluated by Zhou et al. (2015). The light-dark cycles of 1 h-2 h, 1.5 h-1.5 h, 2 h-1 h, 2.5 h-0.5 h, and full light exposure were set every 3 hours at the temperature of 26-30 °C. The effect of light-dark cycle on biomass production and COD removal were evaluated using 1 L glass flasks which were illuminated at 16 Wm^{-2}. The PPB biomass production, COD removal, bacteriochlorophyll and carotenoid pigment production were highest with the light-dark cycles of 2 h-1 h.

2.8. Use of PPB vs microalgae for wastewater treatment

Shoener et al. (2014) indicated that nitrogen and phosphorous removal efficiencies from wastewater using open algal systems ranged from 36% to 87% and 32% to 73% respectively. However, nitrogen and phosphorous removal efficiencies of the photobioreactors using algae were 68%-80% and 85%-90% respectively. Stumm and Morgan (1981) showed that the average COD/N/P uptake ratio for microalgae was 100/16/1. This ratio shows that microalgae is more efficient for nitrogen removal compared to PPB with COD/N/P uptake ratio of 100/6/1 (Hulsen et al.,2016). Hulsen et al. (2016) showed that a hydraulic retention time (HRT) as low as 9 hours can be achieved in municipal wastewater treatment with PPB using a membrane photobioreactor. However, wastewater treatment with algae requires a significantly longer HRT of about 2-10 days (Jorquera et al., 2010; Larsdotter, 2006). Typical HRTs for tubular and flat-plate photobioreactors used for algae growth is 10 days (National-Research-Council, 2012). Posten, (2009) reported that photobioreactors using algae for wastewater treatment are not economic due to high capital and operational costs attributed to the large footprint and long HRTs. However, PPB's application in the wastewater treatment industry offers high COD, N, and P removal efficiencies at low HRTs with a very small footprint (Hulsen et al., 2016).

PPB require lower light intensity in comparison with algae; 16-50 Wm^{-2} (Prasetsan et al., 1993; Zhou et al., 2014; Hulsen et al., 2016) were used for wastewater treatment by PPB versus 100-200 Wm^{-2} used in commercial algal photobioreactors (Gordon and Polle, 2007). Furthermore, the use of infrared LED for PPB reduces the energy consumption per photon by up to 70% compared to the UV-VIS range (200-700 nm) (Bertling et al., 2006). Therefore, the use of PPB in wastewater treatment offers the potential of substantially lower operational costs compared to algae.

2.9. PPB's opportunities

Two PPB's species (*C. vinosum*, and *T. floridana*) were detected from primary and secondary lagoons (70 million gallons volume) which were designed for municipal wastewater treatment (Holm and Vennes, 1970). The PPB enrichment and its growth showed the possibility of wastewater treatment with PPB in the lagoons using sunlight; however, algae and other phototrophic bacteria can grow with a full-spectrum light exposure. UV-VIS filters can be used to

block the spectrums below 790 nm and to prevent the algae growth and the growth of other phototrophic bacteria. A membrane photobioreactor with artificial lights is another possible configuration for municipal wastewater treatment with PPB (Chitapornpan et al., 2013; Hulsen et al., 2016). These reactors consume additional energy for continuous illumination compared to the conventional anaerobic membrane bioreactors (Hulsen et al., 2014).

2.10. Synopsis of the literature review

The majority of prior studies utilized defined PPB cultures, sterilized growth media or synthetic wastewater using batch tests. However, real wastewater and mixed PPB culture must be used to evaluate PPB's performance in wastewater treatment. According to the literature, only one study has been performed to evaluate PPB performance at low temperature and there is no study thus far to determine PPB's Monod kinetic parameters at low temperature. In order to design a photobioreactor for wastewater treatment using PPB at low temperature it is crucial to determine the kinetic parameters involved in the bioreaction. Determination of Monod kinetic parameters is also necessary while the photobioreactor is being operated at a short SRT (< 3 days) due to the sensitivity of the effluent substrate to kinetic parameters.

The previous studies utilized high light intensities (> 50 Wm^{-2}) to prove PPB's viability in wastewater treatment. Using artificial light with high intensities is a major concern in wastewater treatment with PPB as it is neither environmentally friendly nor financially feasible. Hence, it is crucial to decrease energy consumption for the required lighting to enable this technology. Zhou et al. (2014) showed that 40 Wm^{-2} is the threshold light intensity for PPB growth and COD removal, hence, PPB's performance can decrease at higher intensities due to the light inhibition of PPB. Therefore, most previous literature studies could not demonstrate PPB's highest performance due to utilising a higher light intensity than PPB's light threshold of 40 Wm^{-2}. PPB's energy consumption for the photobioreactor's lighting utilised by Hulsen et al. 2016 with an IR light intensity of 50 Wm^{-2} was determined to be 24 kWh/m^3 which is too high for the practical application of the PPB technology.

Zhou et al. (2015) indicated that the highest COD removal, bacteriochlorophyll and carotenoid pigments production were achieved at light-dark cycle of 2 h/ 1 h which can decrease the energy consumption for lighting significantly by up to 30 % compared to full light exposure. Therefore,

the effect of light-dark cycle on PPB performance using photobioreactor is necessary to optimize the PPB technology.

A major challenge in municipal wastewater treatment with PPB is the undesirable COD/N/P ratio. An external COD in the form of ethanol has to be supplemented to the photobioreactor to achieve low effluent TN and TP concentrations (<10 mgNL^{-1} and 10 mgNL^{-1}). However, adding an external carbon source is costly and challenges the economic feasibility of the PPB technology. In industrial wastewater treatment (food processing wastewater), however, an external carbon source is not required due to a high concentration of influent COD (Hulsen et al., 2017). The results of the anaerobic digestion of PPB produced during the treatment of red meat processing wastewater showed that the ratio of the released COD: N: P after 42 days was 100: 2.6: 0.5 (Hulsen et al., 2017). This ratio shows the high potential of PPB fermentate for use an internal COD source rather than any external carbon source in municipal wastewater treatment with PPB, which requires supplementation of COD by an average 300 mgCODL^{-1} as an external COD source to achieve high removals of TN and TP.

2.11. References

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Chapter 3

Low temperature nutrient removal and recovery from municipal wastewater by purple phototrophic bacteria (PPB)[1]

3.1. Introduction

Purple phototrophic bacteria (PPB) are a physiologically versatile group of purple bacteria that can grow under phototrophic conditions as well as in darkness. A variety of PPB were detected in the wastewater treatment plant (WWTP) including *Rba. capsulatus*, *Rps.palustris*, *Rba. sphaeroides*, *Rps. acidophila* and *Rps. crytolactis*. *Rba. capsulatus* is a strain of PPB that can grow under phototrophic conditions with either CO2, organic carbon, or in darkness by respiration, fermentation or chemolithotrophy. *Rba. capsulatus* is probably the most metabolically versatile of all known bacteria (Madigan and gest, 1979). PPB can form dense blooms where the level of sulfide is low or undetectable (Pfenning, 1978a); however, typical PPB can also grow photo-autotrophically (anoxic/light) with low levels of sulfide and H_2 as electron donors (Ehrenreich and widdel, 1994; Brune,1995). PPB are ubiquitous in the natural environment, including wastewater. In a study by Siefert et al., 1978, it was shown that PPB was detected during the activated sludge process. PPB played a minor role compared to heterotrophic bacteria in the activated sludge process, however, PPB can compete with other bacteria in the anaerobic conditions under illumination (Siefert et al., 1978). PPB grow best in media containing readily biodegradable organic compounds such as pyruvate, malate, and ammonia as a source of nitrogen (Sojka, 1978) under phototrophic and anaerobic conditions.

PPB are capable of capturing energy in the form of ATP from light using ATP synthase, light harvesting complexes (LH1, LH2) as well as reaction center in the PPB's cell. The light harvesting complexes of PPB are responsible for the light absorption and the transfer of excited electrons to the photosynthetic reaction center where the energy is trapped in an electron transfer reaction (Law et al., 1999). LH2 contains two rings of bacterochlorophyll B800 BChls and B850 BChls which have prominent absorbance at 800 nm and 850 nm respectively. However, LH1 contains B870 BChls with a prominent absorbance at 870 nm (Koepke et al., 1996). PPB exhibit maximum absorption at 800 nm and 850 nm which is related to LH2 and can be measured according to the optical density (OD) test. Temperatures and light intensities are factors which can affect the performance of LH1 and LH2 which in turn can lead to a lower ATP production and consequently, lower removal efficiencies of COD, N and P from the municipal wastewater. PPB have a broad potential in wastewater treatment attributed to the removal of COD through assimilation (Azad et al., 2001), phosphorous uptake by polyphosphate (polyP) formation (Hiraishi et al., 1991), removal of ammonia via assimilation (Takabatake et al., 2000), as well as removal of nitrate through denitrification (Kim et al., 2004). Valuable products can be simultaneously generated such as polyhydroxybutyrate (Khatipov et al., 1998), bio-hydrogen (Wu et al., 2012) and PPB biomass with high potential utilization as animal feed (Kobayashi and Tchan, 1973) as well as organic fertilizers (Xu, 2001).

Most previous studies (Table 3.1) were conducted at room temperature or above, with long hydraulic retention times (HRT). Previously, PPB have been mostly utilized to treat industrial wastewater (Chitapornpan et al., 2012). However, PPB have been recently employed as a new approach in municipal wastewater treatment to enable nutrient recovery by achieving acceptable effluent COD, TN and TP concentrations below discharge limits (< 50 mgCODL^{-1}, 10 mgNL^{-1} and 1 mgPL^{-1}) (Hulsen et al., 2016). In the above-mentioned study effluent COD, TN, and TP concentrations from municipal wastewater at room temperature were respectively 24±20 mgL^{-1}, 4.8±2 mgL^{-1}, and 0.24±0.1 mgL^{-1}, corresponding to COD, TN and TP removal efficiencies of 97%, 92%, and 97% with ethanol addition of 392 mgCODL^{-1}. The lowest TCOD effluent concentration of 17±24 mgL^{-1} was achieved when the photo bioreactor was operated without an external carbon source. The average BOD effluent was below 10mgL^{-1} (Huslen et al., 2016). Puyol et al., (2017) conducted a series of batch tests using inoculum from the PPB photobioreactor which was operated by Hulsen et al. (2016) to calculate the Monod kinetics. The results of the batch tests with ethanol

showed complete removal of the substrate. Yield coefficient (Y), half velocity constant (K_s), and maximum bacteria growth (μ_{max}) were determined to be 0.63 mgVSS mgCOD^{-1}, 20±4 mgCODL^{-1}, and 1.54 d^{-1}. According to the literature, only one study was carried out to evaluate PPB performance at low temperature (10°C) at HRT of 13 h (Hulsen et al., 2016). Thus, there is a need to address PPB performance at low temperature operating the PHB at HRT of as low as 9 h and to determine Monod kinetics because of the sensitivity of effluent substrate to kinetics at the low SRT (< 3 d) that the photobioreactors typically operate at.

Table 3.1. Selected literature studies on the application of PPB in wastewater treatment

Wastewater	COD removal (%)	N removal (%)	P removal (%)	HRT (d)	Light intensity (lux/ Wm^{-2})	PPB	Temperature (°C)	References
Noodle processing WW	>90	-	-	6-10	-	RP[a] and RB[b]	-	Chiemchaisri et al., 2008
Fish processing WW	43	22	-	3-7	1400 (lux)	RG[c]	32±2	De Lima et al., 2011
Shrimp-blanching water and tuna condensate	86	-	-	5	3000 (lux)	RG[c]	30	Prasetsan et al., 1993
Olive mill WW	33	-	-	-	200 (Wm^{-2})	RS[d]	32	Eroglu et al., 2010
Food processing WW	58	-	-	10	270 (Wm^{-2})	Mixed	22	Chitapornpan et al., 2012
Swine WW	50	-	58	6	500-5000 (lux)	RP[a]	20-50	Kim et al., 2004
Dairy WW	93	-	-	3	1300 (Wm^{-2})	Mixed	22	Kaewsuk et al., 2010
Municipal WW	91	81	82	< 1	50 (Wm^{-2})	Mixed	22	Hulsen et al., 2016
Municipal WW	87	77	98	< 1	50 (Wm^{-2})	Mixed	10	Hulsen et al., 2016

[a] *Rhodopseudomonas palustis,* [b] *Rhodobacter blasticus,* [c] *Rubrivivax gelatinosus,* [d] *Rhodobacter sphaeroides*

In this study, the application of PPB for municipal wastewater treatment at low temperature was evaluated by conducting a series of batch tests and continuous-flow membrane photobioreactor experiments. The key objectives are; (a) assessment of PPB performance at low temperature based on photo bioreactor treatment efficiency and key Monod kinetics parameters (b) PPB enrichment and simultaneous removal of soluble biodegradable and inert COD, TN and TP to below target limits of 50, 10, and 1 mgL^{-1};(c) determination of the optimum HRT at 22°C and 11°C; and (d) comparison of microbial growth at 22°C and 11°C.

3.2. Materials and methods

3.2.1. Raw wastewater

Municipal wastewater was collected from the Greenway treatment plant in London, Ontario and was stored immediately in a cold room at a temperature of 4°C. The wastewater was permitted to settle for a period of 2 hours and was subsequently used as the primary effluent wastewater. The municipal wastewater characteristics are summarized in Table 3.2.

Table 3.2. Municipal wastewater characteristics

Parameters	T=22°C	T=11°C
	Values	Values
TCOD (mg/L)	370 ± 60 (n=35)	540 ± 20 (n=16)
SCOD (mg/L)	200 ± 50 (n=35)	290 ± 30 (n=16)
TSS (mg/L)	160 ± 30 (n=35)	240 ± 20 (n=16)
VSS (mg/L)	110 ± 22 (n=35)	160 ± 20 (n=16)
TN (mg/L)	48 ± 11 (n=35)	56 ± 7 (n=16)
TKN (mg/L)	47 ± 11 (n=35)	55 ± 7 (n=16)
NH$_4$-N (mg/L)	34 ± 11 (n=35)	39 ± 8 (n=16)
NOx(mg/L)	0.78 ± 0.3 (n=35)	0.88 ± 0.2 (n=16)
TP (mg/L)	6.6 ± 2 (n=35)	8.2 ± 1 (n=16)
SP (mg/L)	3.7 ± 1.5 (n=13)	4.3 ± 0.6 (n=16)

3.2.2. PPB enrichment

PPB enrichment was carried out using a series of fed-batch tests at room temperature. Raw municipal wastewater was anaerobically illuminated at the IR light intensity of 50 Wm^{-2}. The influent SCOD, NH_4, and PO_4 concentrations were 410 mg/L, 29.3 mg/L, and 3.9 mg/L respectively. Ethanol was dosed at 100 mgCOD/L as an external carbon source. The batch tests were conducted using two bottles with a working volume of 250 mL. The bottles were continuously mixed at 100 rpm. The samples were allowed to settle for 2 hours on days 4, 8, 12 16, and 20, 200 mL of the supernatant were discarded and replaced with 200 mL fresh raw wastewater that were added to the 50 mL PPB inoculum which remained in the bottles after the settlement period. The SCOD, NH_4, and PO_4 concentrations were measured every 4 days to evaluate the PPB growth and COD, N, and P removal from the municipal wastewater.

3.2.3. Anaerobic membrane photobioreactor configuration and operation

As shown below in Figure 3.1, a 2 L rectangular acrylic photobioreactor (Hulsen et al., 2016) with a submerged flat sheet membrane (Kubota submerged membrane made from polyolefin) consisting of 0.12 m^2 surface area and 0.45 µm pore size was illuminated under infrared LED lamps at 50 Wm^{-2} (96 IR LED for Night Vision Camera). The maximum operating flux of the membrane was 1.9 L/m^2h^1. The height, length and width of the photobioreactor were 40, 34, and 2 cm respectively. The photobioreactor was continuously mixed with internal gas from the headspace of the reactor using a vacuum pump (Cole Parmer, Air Cadet, R-K 07530-85). The air is carried through the vacuum pump to the reactor via a diffuser (Magi Deal Fish Tank Pond Pump Air Stone Bubble Disk Tube Aerator Aquarium- 12 inch) placed at the bottom of the reactor. The reactor was fed with raw wastewater (primary effluent) using a peristaltic pump (Cole Parmer, 7520-67 Masterflex Console Drive Peristaltic pump).

Figure 3.1. Photobioreactor (PHB) configuration

In period 1, the photobioreactor was started using a 200 mL PPB inoculum from the fed-batch tests at 22°C and at the IR light intensity of 50 Wm^{-2} (same conditions as in period 1). Similar batch tests were carried out using a refrigerator at 11°C to enrich the PPB inoculum for period 7, where the PHB was operated at 11°C. These fed-batch tests were conducted for the duration of 3 weeks (Appendix A).

The average photobioreactor temperature and pH were respectively 22°C and 7.2 during this entire experimentation phase (6 periods). Experimental analysis was conducted once every three days.

The sludge withdrawal was collected from the bottom of the photobioreactor to control sludge retention time (SRT). The PHB was opened three times, on day 78, 117 and 147 to remove the PPB biofilm from the membrane and the reactor's walls to minimize the biological impact of the PPB biofilm formed on the membrane. COD, nitrogen, and phosphorous steady state mass balances were evaluated at an SRT of 3 days. The mass balance was calculated according to equation 1.

$$\text{Mass closure (\%)} = \frac{\text{Effluent conc (mgL}^{-1}) \cdot \text{Effluent volume (Ld}^{-1}) + \text{Sludge conc (mgL}^{-1}) \cdot \text{Sludge volume (Ld}^{-1})}{\text{Influent conc (mgL}^{-1}) \cdot \text{Influent volume (Ld}^{-1})} \quad (1)$$

A summary of PPB operating conditions is shown in Table 3.3. In period 1 (day 1-18), HRT was set to 36 h with no biomass withdrawal and without the addition of ethanol. Ethanol was dosed at 290 ± 30 (mgCODL^{-1}) in period 2 (day 18-27) when HRT was 36 h with no biomass withdrawal. In period 3 (day 27-57), HRT and SRT were adjusted respectively to 18 h and 3 d without the addition of an external carbon source. HRT and SRT were maintained at 18 h and 3 d in period 4 (day 57-78) and ethanol was added to the reactor at a concentration of 285 ± 40 (mgCODL^{-1}). In period 5 (day 78-102) when ethanol was withdrawn, HRT decreased to 9 h while SRT was maintained at 3 d. Ethanol was dosed at 310 ± 20 (mgCODL^{-1}) in period 6 (day 102-117) where HRT and SRT were maintained at 9 h and 3 d respectively.

Table 3.3. Summary of operational parameters

Period	HRT (h)	SRT (d)	Ethanol (mg/L)	Temp (°C)
Period 1 Day 0-18	36	–	–	22
Period 2 Day 18-27	36	–	290	22
Period 3 Day 27-57	18	3	–	22
Period 4 Day 57-78	18	3	285	22
Period 5 Day 78-102	9	3	–	22
Period 6 Day 102-117	9	3	310	22
Period 7 Day 117-132	36	3	400	11
Period 8 Day 132-147	18	3	300	11
Period 9 Day 147-165	9	3	300	11

Following the optimization of the HRT, the PHB was used to investigate performance at low temperature while maintaining the optimum HRT at 9 h. As shown in Table 3, the PHB was operated at 11°C for periods 7-9. A refrigerated recirculating water bath was employed in order to

maintain the PHB temperature at 11±1°C (PolyScience MX 7L Refrigerated Bath Circulator). The PHB's temperature was measured on a daily basis. The PHB's pH level remained constant at 7.3 throughout the experiment. The PPB inoculum for the continuous-flow PHB was grown in a batch test at the same temperature as the reactor (11±1°C). The PHB was operated at three different HRTs (36 h, 18 h and 9 h) while the SRT was controlled at 3 d in each period.

3.2.4. Analytical methods

Various water quality parameters were continuously measured for influent and effluent samples. Total chemical oxygen demand (TCOD), soluble chemical oxygen demand (SCOD) were measured using HACH methods (HACH Odyssey DR 2800). Total and soluble phosphorous, total nitrogen and total kjeldahl nitrogen (TKN) were determined using HACH methods. Total suspended solids (TSS) and volatile suspended solids (VSS) were determined by drying at 105°C for 2 hours and at 550°C for 20 minutes respectively following standard methods (APHA, 1998). All soluble samples were obtained by filtration through a 0.45 µm pore size (GN Metricel, Membrane Disc Filters, Pall Laboratory).

3.2.5. Microbial growth

The wavelength scan (optical density) test was employed during periods 4 and 8 at temperatures of 22°C and 11°C to provide insight into PPB enrichment. This analysis was conducted using DR 3900 spectrophotometer to show the absorbance behaviour of the PHB samples and adaptation to PPB strains. The profile of absorption spectra in most PPB follow a specific trend with three peaks near infrared region at 800 nm and 860 nm and at 590 nm due to the presence of bacteriochlorophyll pigments and two peaks at 460 nm and 488 nm due to the presence of carotenoid pigments in PPB's cell.

Light harvesting complexes (LH1 and LH2) play a significant role in the absorption of light and energy in the PPB structure. LH1 exists in all PPB while most PPB species contain LH2. LH2 absorbs a higher level of energy compared to LH1; consequently, the absorption profile of LH2 is crucial in determining which wavelength has the highest absorbance near infrared regions. *Rps. palustris*, *Rps. cryptolactis*, *Rba. capsulatus* and *Rps acidophila* are PPB species that contain both LH1 and LH2 which are mainly made by bacteriochlorophyll a. The spectrum of the two major

bands in the highest absorption range for *Rps. cryptolactis* are 803nm and 858nm with the ratio of optical density at 858 nm which is typically 1.2 times that at 803 nm. *Rps. acidophila* peaks are at 801nm and 856nm with the ratio of optical density at 856 nm which is typically 1.3 times that at 801 nm. The spectrum of the two major bands in the highest absorption for *Rba. capsulatus* and *Rps. palustris* are respectively at 802 nm and 859 nm as well as 803 nm and 853 nm (Georgakopoulou et al., 2002).

3.2.6. Monod kinetic model

The Monod model was used to determine the PPB kinetics. In order to simulate this model, MATLAB (MATLAB R2017a, The MathWorks Inc., Natick, MA) was used to determine the parameters μ_{max}, K_s, K and K_d using nonlinear curve fitting (lsqcurvefit). A system of differential equations (equations 2, and 3) with variables (X(t) and S(t)) and parameters (μ_{max}, K_s, K and K_d) was solved using ODE45. The curve fitting method was used to evaluate the best fit of parameters to Monod equations considering the experimental data X(t) and S(t) which were measured at time intervals of 1 h using batch tests at both 22°C and 11°C.

According to the model, substrate (S) and cell (X) concentrations were determined by solving the differential equations using ODE45 based on estimated Monod parameters and the initial substrate and cell concentrations at time zero.

$$\frac{dX}{dt} = \frac{\mu_{max}\,SX}{K_S + S} - K_dX \tag{2}$$

$$\frac{dS}{dt} = \frac{1}{Y}\frac{\mu_{max}SX}{K_S + S} \tag{3}$$

The batch tests were conducted to model Monod kinetics using 50 mL PPB inoculum from PHB at HRT of 9 h (periods 6 and 9) that was mixed with 150mL fresh wastewater. Ethanol was dosed at 100 mgCODL^{-1}. 10 mL samples were collected hourly for a period of ten hours to measure the COD and VSS concentrations. The initial COD, N, and P concentrations of the wastewater and inoculum are summarized in Table 3.4.

Table 3.4. COD, N, and P concentration of the inoculum and fresh wastewater

Parameters	Batch tests at 22°C		Batch tests at 11°C	
	Wastewater (150 mL)	PPB inoculum (50 mL)	Wastewater (150 mL)	PPB inoculum (50 mL)
TCOD (mg/L)	365	1350	405	1040
SCOD (mg/L)	160	44	189	61
TN (mg/L)	42.4	85	43.2	69.6
NH4 (mg/L)	30	4.2	32	5.9
PO4 (mg/L)	3.2	0.75	3.4	0.88
TP (mg/L)	7.28	16.8	7.7	10.8
Ethanol (mgCOD/L)	100			
Total volume (mL)	200		200	

3.3. Results and discussion

3.3.1. PPB enrichment

The SCOD, NH_4, and PO_4 removal efficiencies on day 8 were 12%, 9%, and 15% respectively. The low removal efficiencies were due to the inadequate PPB culture. Based on Figure 3.2, the effluent SCOD, NH_4, and PO_4 concentrations on day 20 were 133, 10, and 0.9 mgL^{-1} respectively, corresponding to the SCOD, NH_4, and PO_4 removal efficiencies of 68%, 65%, and 77%. The increase in the SCOD, N, and P removal efficiencies during days 8-20 showed the simultaneous PPB growth and substrate removal from municipal wastewater.
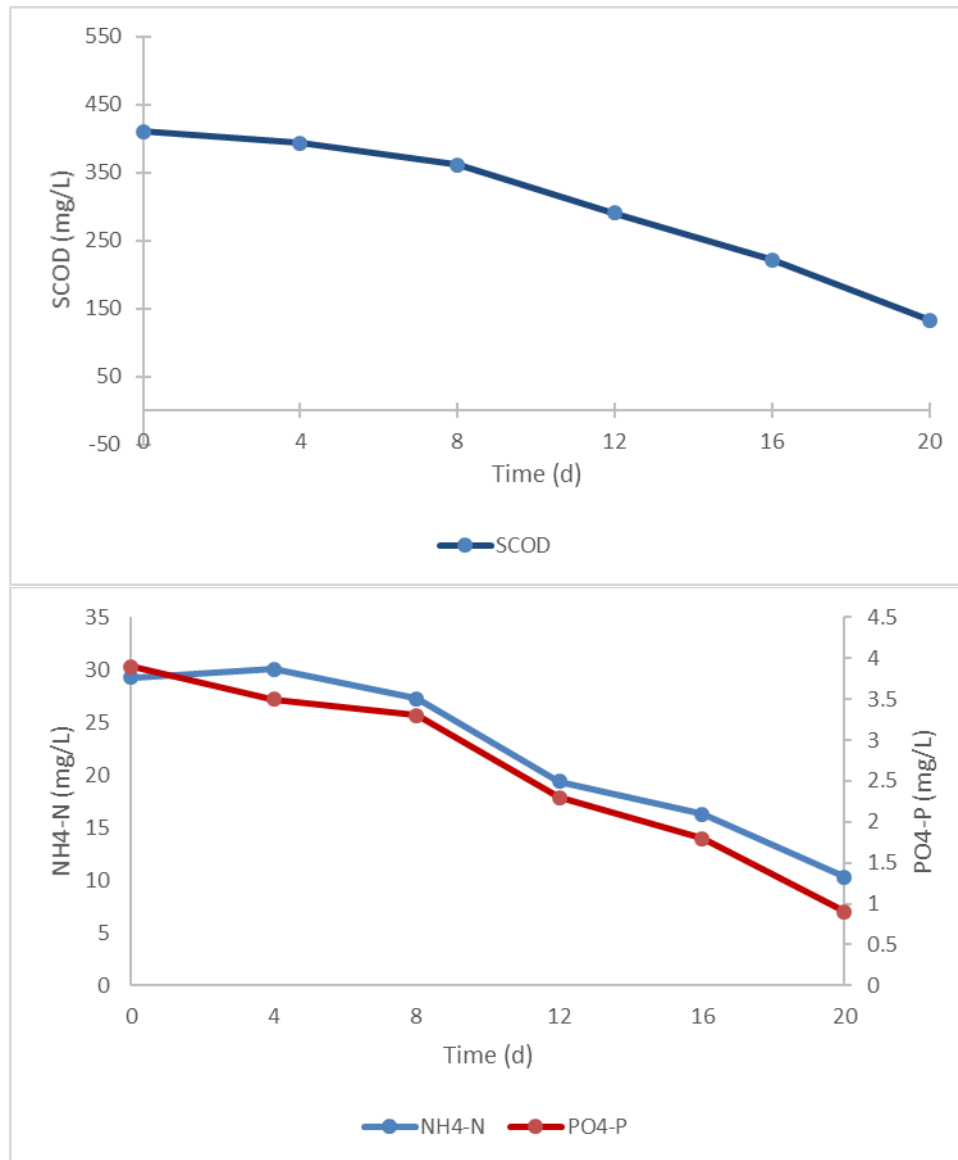
Figure 3.2. The SCOD, NH₄, and PO₄ concentrations in fed-batch tests

3.3.2. Photobioreactor operation and optimization at 22°C

The photobioreactor (PHB) effluent TCOD, TN, and TP concentrations are summarized in Table 3.5. Target effluent COD, TN, and TP concentrations were set at 50,10, and 1 mgL^{-1} respectively, typical of biological nutrient removal plants. Based on the typical range of soluble inert COD in raw municipal wastewater of 5%-10% of TCOD ASM2 (Henze et al.,1995), using an average fraction of 10% or 37 mgCODL^{-1}, effluent soluble BOD₅ is estimated at mgL^{-1}.

Table 3.5. Startup (periods 1 and 2) and steady-state (periods 3,4,5, and 6) effluent characteristics (values in mgL^{-1})

Periods	TCOD (mg/L)	TN (mg/L)	TP (mg/L)	NH4 (mg/L)	Nox (mg/L)	MLSS (mg/L)	MLVSS (mg/L)	n[a]
1	190±35	37±5	1.5±0.4	29±7	0.5±0.06	1270±400	1080±370	6
2	70±10	8±9	0.9±0.1	2±1	0.3±0.06	1630±130	1390±110	3
3	44±5	30±6	1.3±0.3	25±7	0.3±0.08	710±60	630±60	10
4	46±2	5±1	0.8±0.1	3±1	0.2±0.05	850±50	710±35	10
5	44±2	30±2	1.3±0.1	24±5	0.3±0.04	1300±30	1090±35	8
6	46±3	5±1	0.6±0.2	3±1	0.2±0.07	1420±25	1180±25	7

[a] Values represent average ± standard deviation, and n is the number of samples

Period 1:

The PHB TCOD, TN, and TP removal efficiencies are demonstrated in Figure 3.3. During the start-up (18 days), HRT was set to 36 hours and PHB worked without biomass withdrawal. This period was utilized to provide the PPB with the desirable conditions (anaerobic phototrophic conditions) that led to the rapid growth and color change of PPB to purple. TCOD and TN removal efficiencies fluctuated due to the inadequate PPB biomass with average TCOD and TN concentrations of 190 mgL^{-1} and 37 mgL^{-1}, while the average effluent phosphorous concentration was 1.5 mgL^{-1} (corresponding to 82% TP removal efficiency).

Period 2:

During this period, ethanol was dosed at 290 mgCODL^{-1} as the external carbon source. Higher removal efficiencies (89% TCOD, 91%TN and 89%TP) were achieved as a result of the adequate PPB biomass and ethanol dosage. The effluent concentrations of TN and TP were respectively 8 mgL^{-1} and 0.9 mgL^{-1} which met the target limits (< 10 mgNL^{-1} and 1 mgPL^{-1}) and the average effluent TCOD concentration decreased to 70 mgL^{-1}.

Period 3:

Once the ethanol was withdrawn and HRT as well as SRT were set to 18 hours and 3 days respectively, the effluent nitrogen considerably increased to 30 mgL^{-1} due to the lack of carbon, meanwhile, effluent phosphorous slightly rose to 1.3 mgL^{-1} (corresponding to 81% TP removal efficiency). The average effluent TCOD concentration was 53 mgL^{-1} corresponding to TCOD removal efficiency of 86%. However, the high effluent nitrogen concentration showed the need for supplementing the soluble COD in order to achieve the required target limit for the effluent nitrogen concentration.
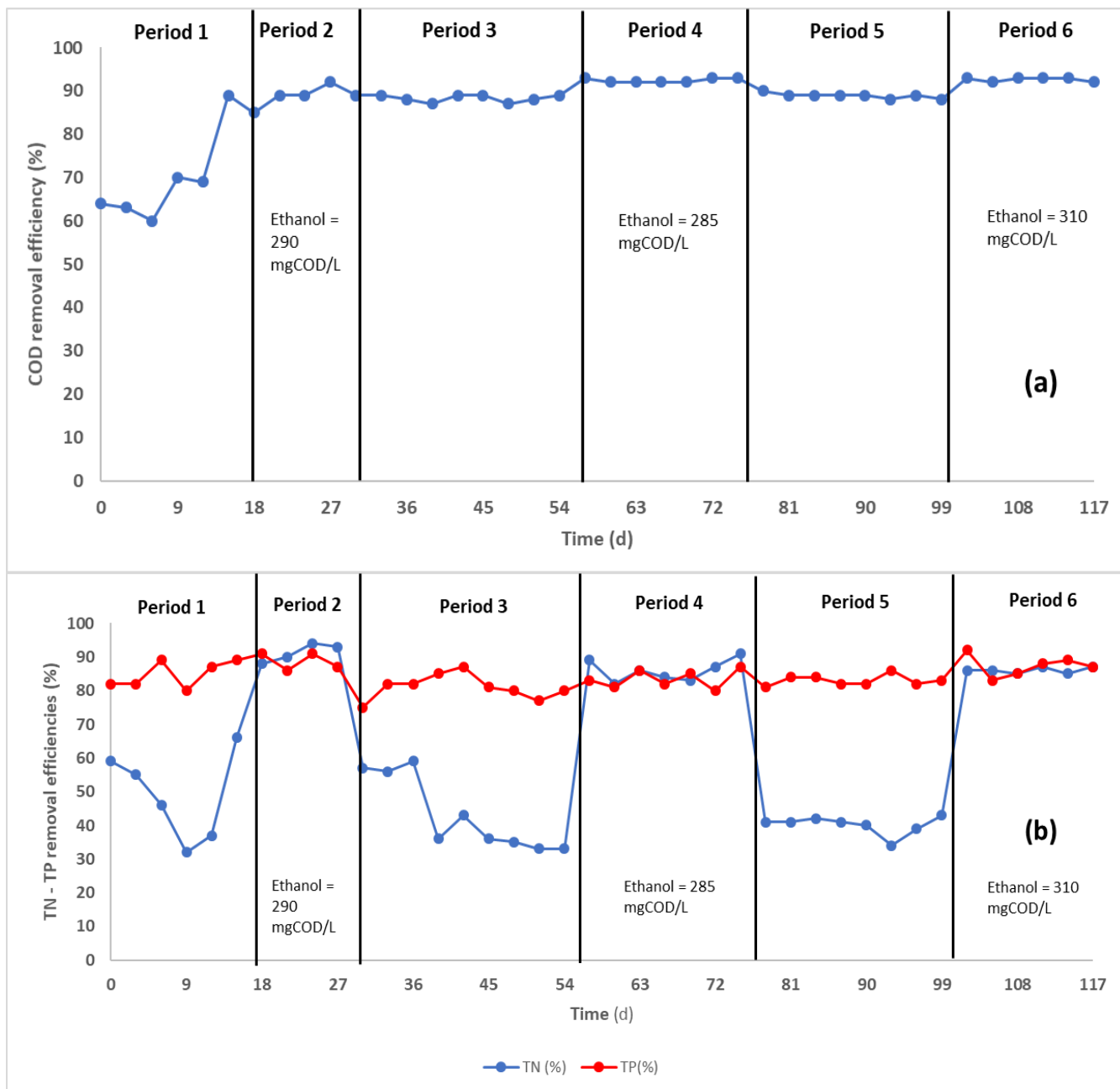
Period 4:

HRT and SRT were maintained at 18 hours and 3 days respectively and the ethanol was dosed at 285 mgCODL^{-1}. PPB performance immediately improved when ethanol was added to the PHB, especially for nitrogen removal. The TCOD, TN, and TP effluent concentrations were respectively 49 mgL^{-1}, 5.5 mgL^{-1} and 0.8 mgL^{-1}, corresponding to TCOD, TN, TP removal efficiencies of 92%, 86%, and 84% respectively.

Period 5:

When the HRT decreased from 18 to 9 hours, the SRT was maintained at 3 days, and the ethanol was withdrawn, the PHB performance was similar to period 3. The average TCOD, TN and TP effluent concentrations were respectively 46 mgL^{-1}, 29 mgL^{-1} and 1.3 mgL^{-1}, corresponding to TCOD, TN, TP removal efficiencies of 89%, 40%, and 83% respectively.

Period 6:

HRT and SRT were kept respectively at 9 hours and 3 days, ethanol was added to the photobioreactor at a dose of 310 mgCODL^{-1}. The average effluent COD, N and P remained below the target limits (46 mgCODL^{-1}, 5.9 mgNL^{-1} and 0.6 mgPL^{-1}) corresponding to TCOD, TN, and TP removal efficiencies of 93%, 86% and 88% respectively. The high efficiency of this period indicated that within the range of 9 h to 36 h, the optimum HRT is 9 hours.

Figure 3.3. TCOD (a), TN, and TP (b) removal efficiencies versus time

Influent and effluent COD, N, and P concentrations are displayed in Figures 3.4, 3.5, and 3.6 respectively. In periods 4, and 6, when the PHB was operated with enough carbon, effluent NH_4, and NO_x concentrations were respectively 3 ± 1 (mgL^{-1}) and 0.18 (mgL^{-1}) corresponding to the ammonia removal efficiency of 89%. Therefore, there is no nitrification and N, and P are only

removed by biomass synthesis. It is important to note that when the external carbon source was withdrawn, the ammonia removal efficiency significantly decreased to 36%.
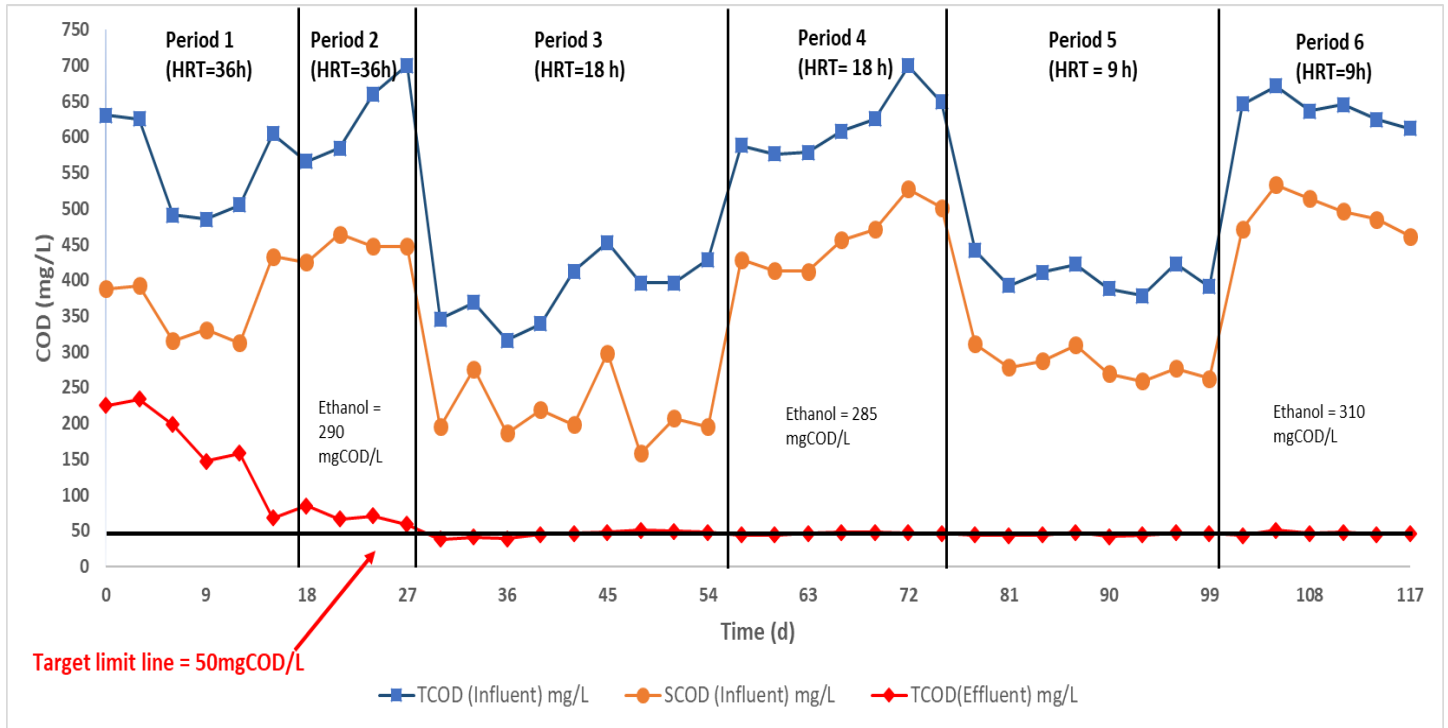

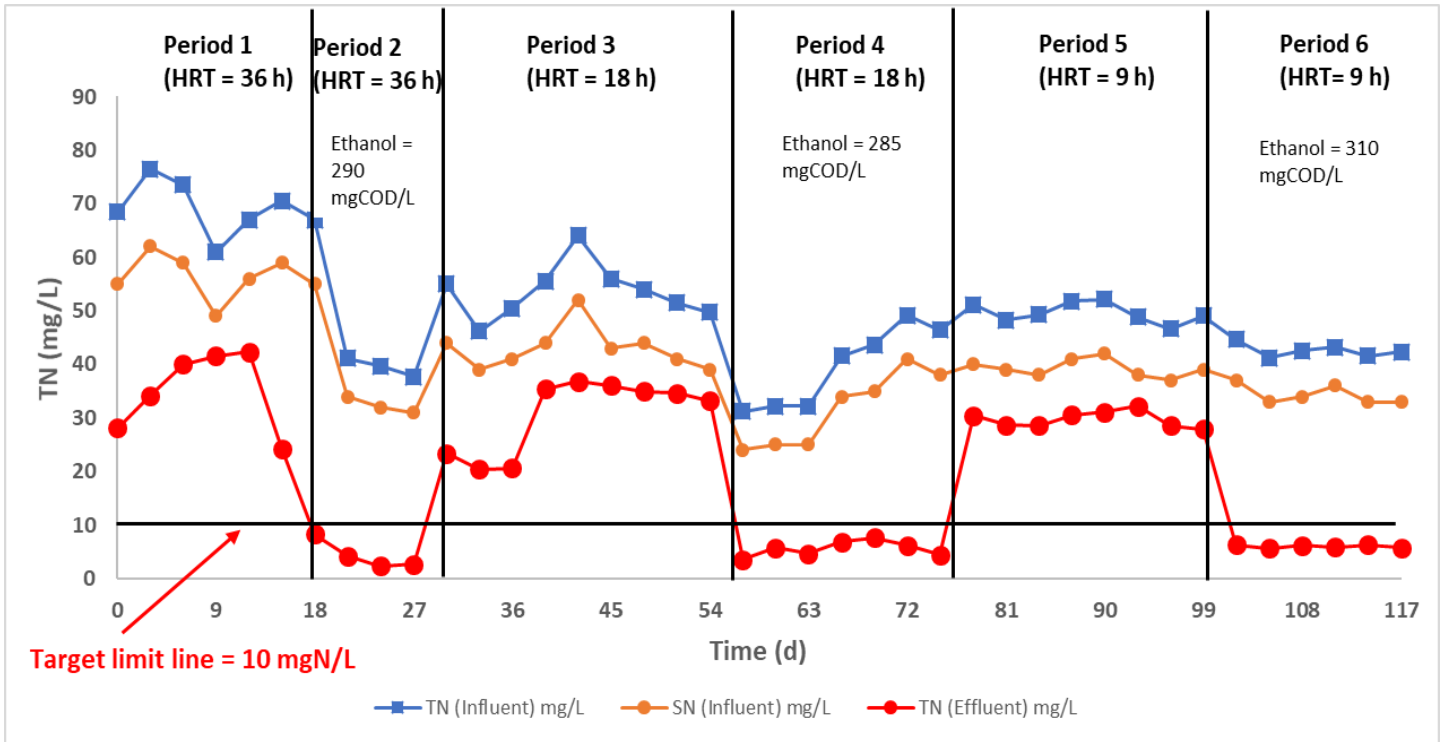**Figure 3.4. Influent and effluent COD concentrations at 22°C**

Figure 3.5. Influent and effluent N concentrations at 22°C
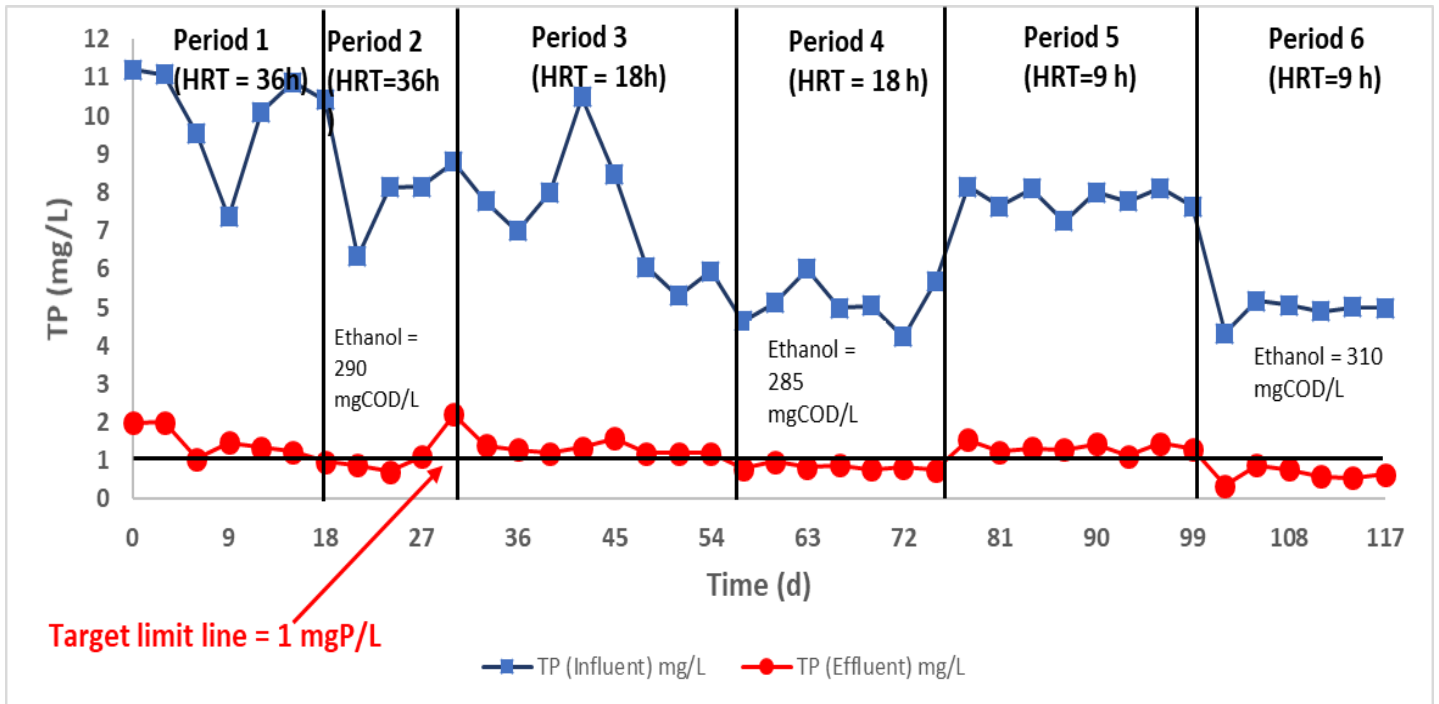

Figure 3.6. Influent and effluent P concentrations at 22°C

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Steady state mass balances at HRTs of 18 h (periods 3 and 4) and 9 h (periods 5 and 6) respectively showed COD mass closure of 88%±5% and 84%±3%, TN mass closure of 88%±6% and 82%±3% as well as TP mass closure of 85%±4% and 80%±1%. COD: N: P uptake ratio was 100: 6.1±0.8: 1.3±0.4. The average COD, nitrogen and phosphorous contents of the PPB biomass as a percentage of dry organic weight were respectively 163%±8%, 10.7%±0.5%, and 2%±0.1%. The average VSS to TSS ratio was 83%. Hulsen et al. (2016) showed that the average COD: N: P ratio for PPB of 100: 6: 1. By comparison with the above-mentioned nutrients uptake, the nitrogen content of PPB is consistent with Hulsen et al. (2016) while P uptake is 30% higher than average.

3.3.3. Municipal wastewater treatment with PPB at 11°C

The PHB effluent characteristics throughout the 48 days of operation at 11°C are summarized in Table 3.6 while the temporal variations for COD, N, and P are depicted in Figures 3.7, 3.8, and 3.9 respectively. The average removal efficiencies of TCOD, TN, and TP were respectively 93%, 88% and 91% during the first 30 days (period 1 and 2), when the HRTs were 36 h and 18 h. Effluent TCOD, TN, and TP concentrations slightly increased when the HRT was reduced to 9 h with removal efficiencies of 91%, 80% and 80% respectively. Effluent TCOD concentrations were slightly more than the target limit during periods 7, 8, and 9, whereas effluent TN and TP concentrations were below the target limits except for period 3. Steady state mass balance for periods 8, and 9 demonstrated TCOD, TN, and TP recoveries of 80%±2%, 83%±1%, and 86%±2% respectively.

Table 3.6. Steady state effluent characteristics at 11°C

Periods	TCOD (mg/L)	TN (mg/L)	TP (mg/L)	NH4 (mg/L)	Nox (mg/L)	MLSS (mg/L)	MLVSS (mg/L)	n^a
7	62±1	7±1	0.6±0.1	4±1	0.4±0.08	440±25	360±20	6
8	63±2	7±1	0.7±0.1	4±1	0.4±0.07	715±30	600±20	5
9	70±2	10±1	1.2±0.1	6±1	0.5±0.08	1260±45	1040±30	6

[a] Values represent average ± standard deviation, and n is the number of samples
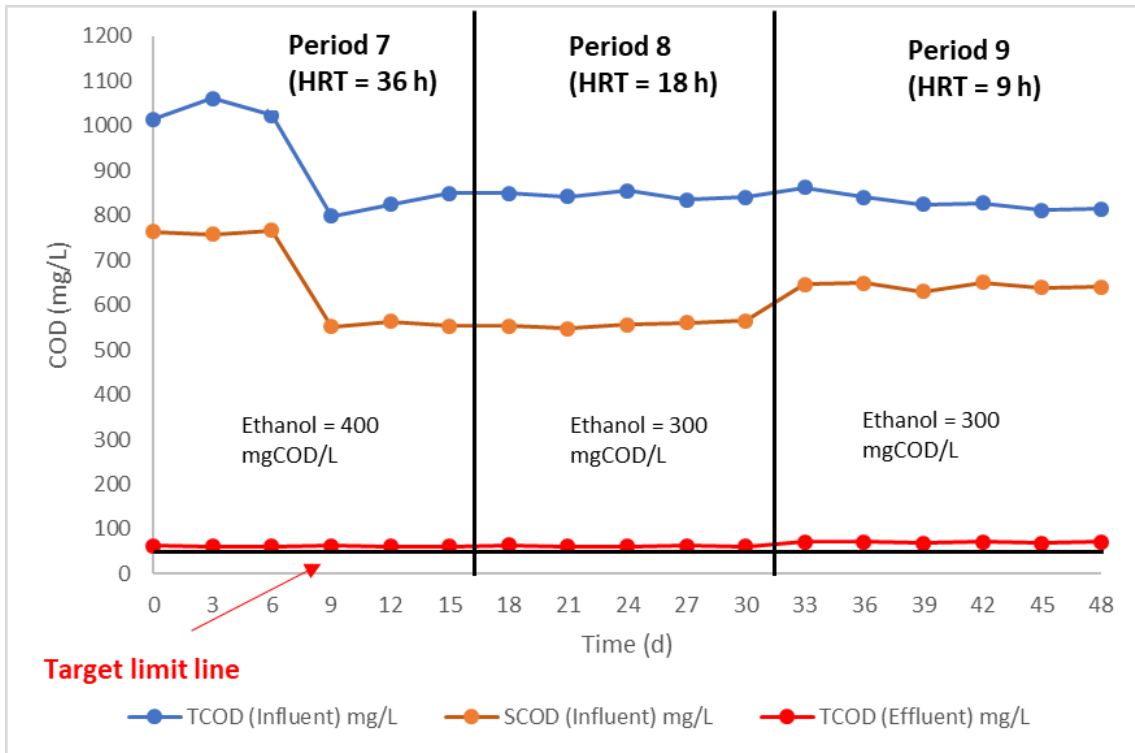
Figure 3.7. Influent and effluent COD concentrations at 11°C
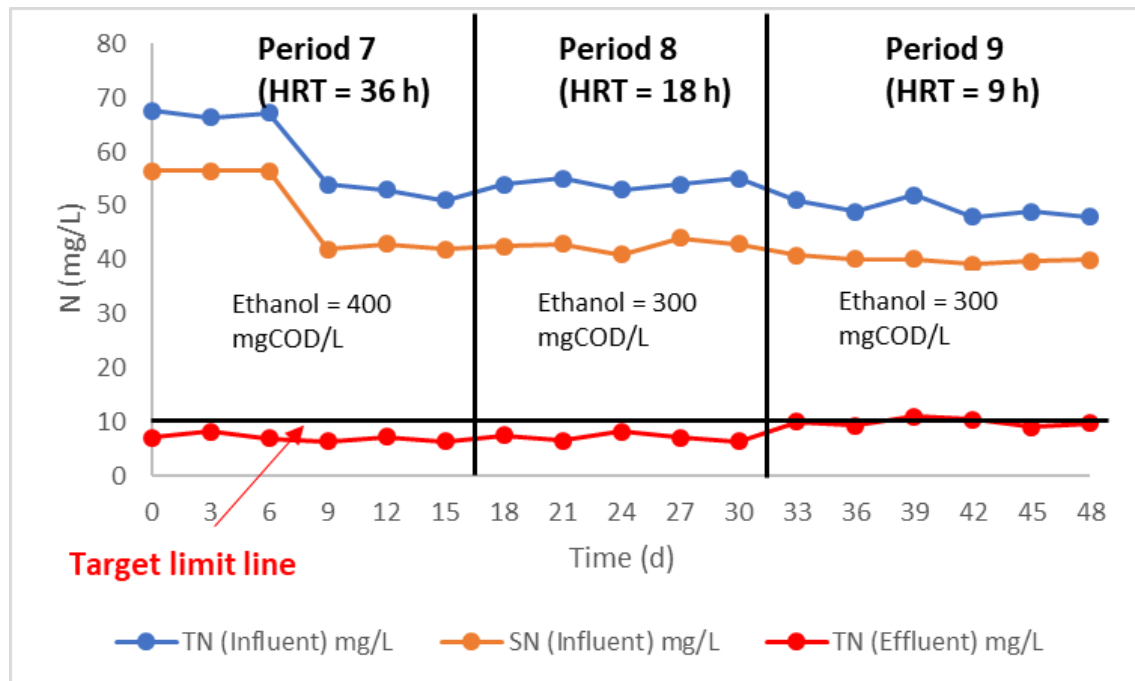
Figure 3.8. Influent and effluent N concentrations at 11°C

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Figure 3.9. Influent and effluent P concentrations at 11°C

3.3.4. Microbial growth

The results of OD tests at 22°C and 11°C showed three peaks at 590 nm, 800, and 860 nm (bacteriochlorophyll) and two peaks at 460 nm, and 488 nm (carotenoid) which are depicted in Figure 3.10. During periods 4, and 8 where the PHB was operated at HRT of 18 h and SRT of 3 d, the optical density was measured at 22°C (e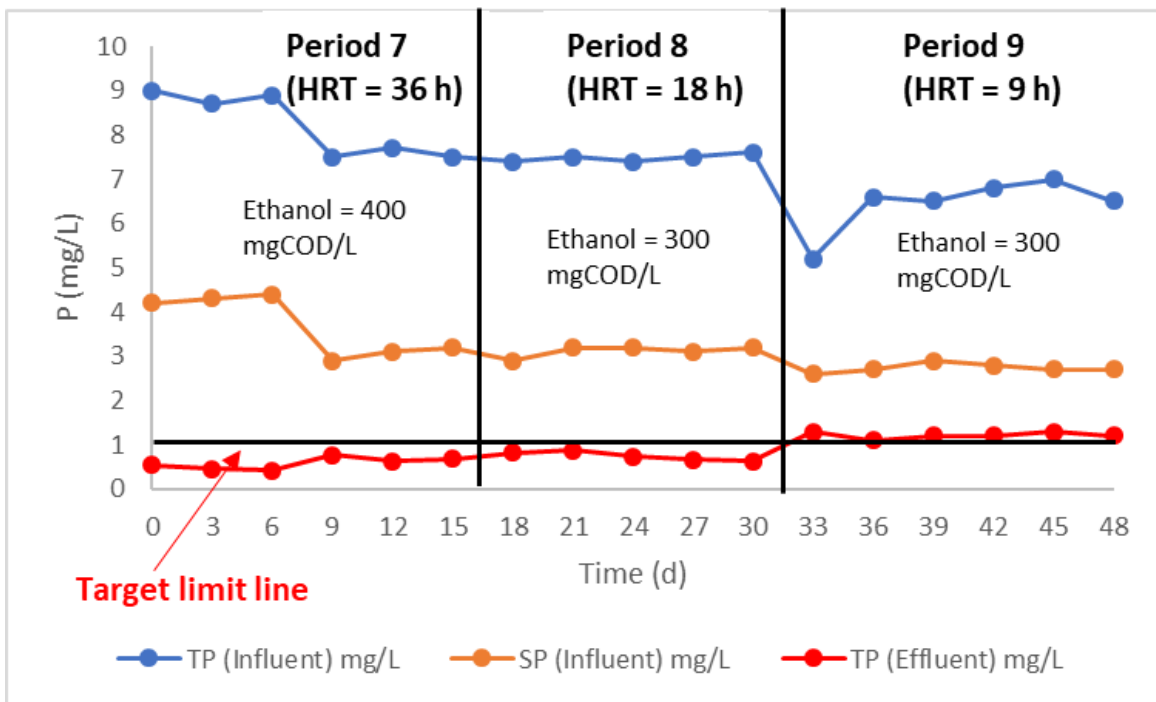thanol dose of 285 mgCODL^{-1}) in period 4 and at 11°C (ethanol dose of 300 mgCODL^{-1}) in period 8.The optical density (OD) test results of samples taken from the PHB in both periods 4 and 8 demonstrated two peaks at 800 nm and 860 nm with the ratio of 1.2 $\frac{\text{OD at 858 nm}}{\text{OD at 800 nm}}$. These results are in consistence with PPB absorption characteristics which exhibit a similar trend with peaks at approximately 800 nm and 860 nm with the ratio between 1.1 and 1.4 $\frac{\text{OD at 858 nm}}{\text{OD at 800 nm}}$. These two peaks confirm the presence of bacteriochlorophyll pigments (B800, and B850) in PPB, known as LH2. The ratio of OD (850 nm) and OD (800 nm)

depends on light intensity and temperature, since both of these factors can affect light harvesting complexes (LH1 and LH2) of PPB.
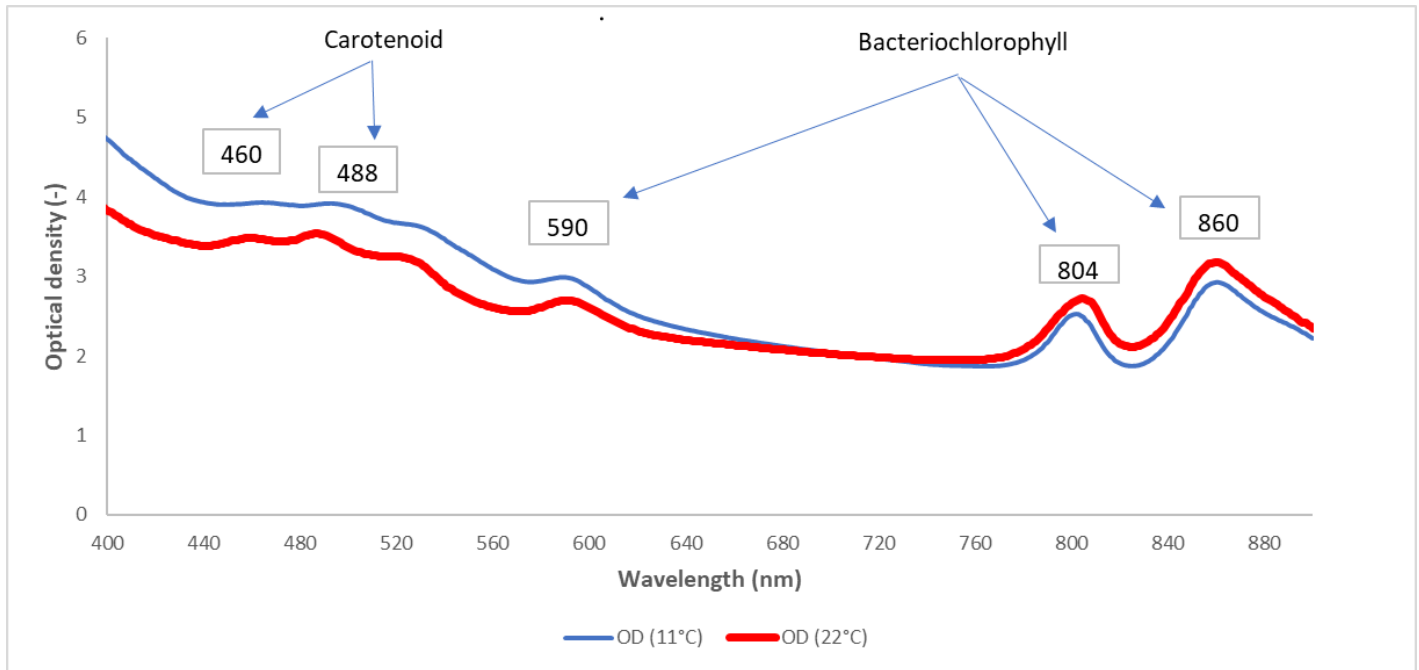
Figure 3.10. PPB wavelength scan (full-spectrum OD) test at 22°C and 11°C

3.3.5. Monod kinetic model

Monod kinetics parameters in the batch tests were determined by using nonlinear curve-fitting (lsqcurvefit) of the experimental data (X and S) in Matlab (MATLAB R2017a, The MathWorks Inc., Natick, MA). K_s, K, Y, and K_d at 22°C were determined to be 20 ± 2 mgCODL^{-1}, 1.9 ± 0.2 mgCOD(mgVSSd)$^{-1}$, 0.47 ± 0.05 mgVSS mgCOD^{-1}, and 0.07 ± 0.01 d^{-1} respectively. According to Figure 3.11, the average percentages of the discrepancy between experimental data and Monod fit for substrate (S) removal and cell (X) growth were 15% and 1.4% respectively, demonstrating the accuracy of the model parameters.
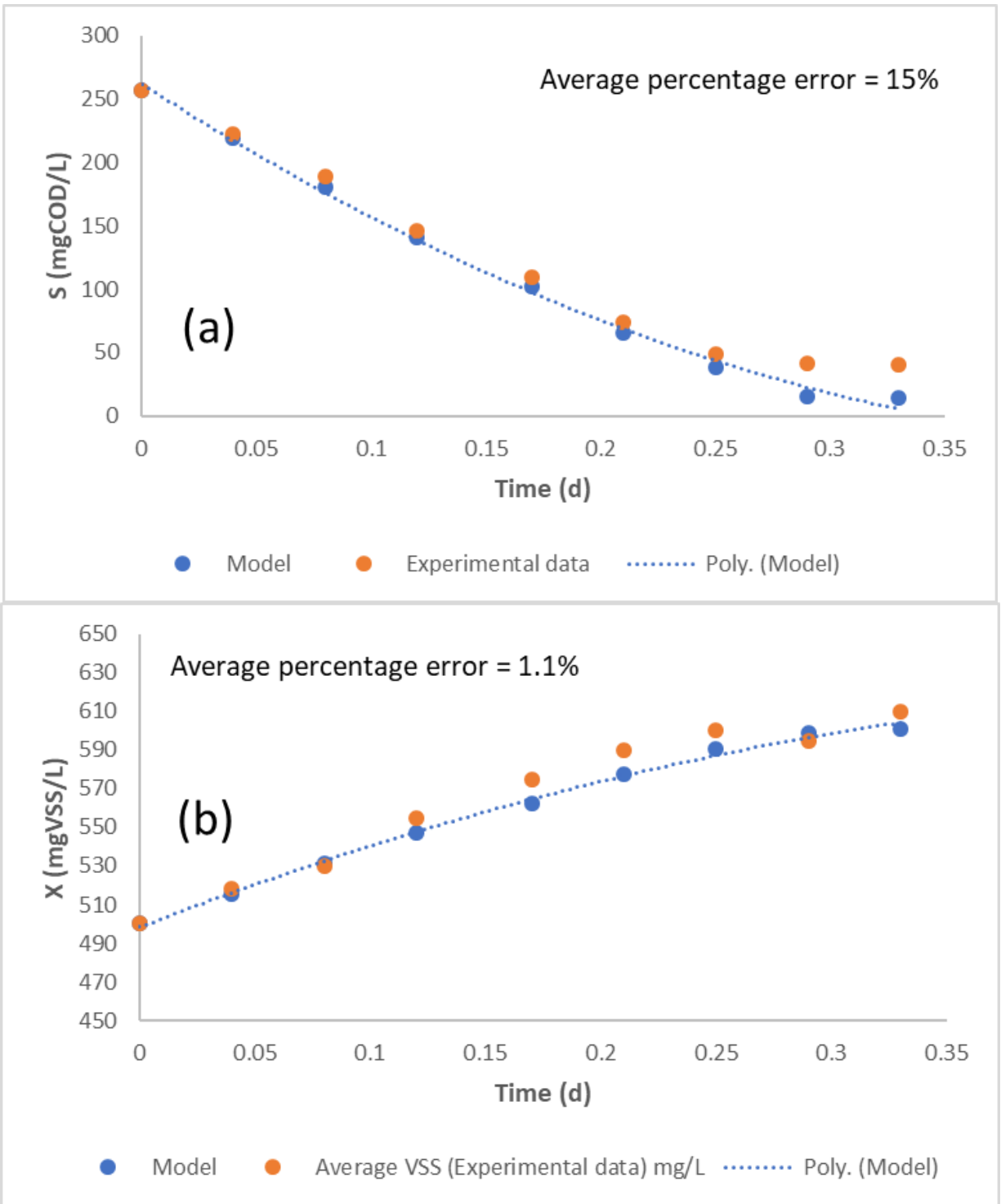
Figure 3.11. Monod fit and experimental data for substrate removal (a) and cell growth (b) at 22°C

According to equation 4, the effluent substrate concentration in a continuous-flow reactor is the function of SRT, K_s, K_d, K and Y. The PHB effluent substrate concentration using the above-mentioned kinetic parameters was calculated to be 17 $mgCODL^{-1}$ based on equation (4), while experimental analysis showed the average effluent of 45 $mgCODL^{-1}$. The soluble microbial products (SMPs) was estimated to be 15 $mgCODL^{-1}$ using equations 5, and 6 (Rittmann et al., 1987). It must be asserted that although the biokinetics of aerobic and anaerobic systems are very different, the findings of Rittmann et al. (1987) suggest that the kinetic parameters for SMPs i.e. K_1, K_2, K_{3m} are similar to both aerobic and anaerobic systems. This analysis indicated that the concentration of inert soluble COD in the wastewater collected from the Greenway treatment plant is 13 $mgCODL^{-1}$ consistent with Gupta et al. (2018) who observed an inert soluble COD of 14 $mgCODL^{-1}$ in the same wastewater.

$$S = \frac{K_s\,(1 + K_d\,.SRT)}{SRT\,(\mu_{max} - K_d) - 1} \tag{4}$$

$$P = \frac{P_0}{1 + \dfrac{K_{3m}.X_a.\Theta_c}{P_0}} \tag{5}$$

$$P_0 = -K_1.(S_0 - S) + K_2.X_a.\Theta_c \tag{6}$$

Where, S_0, and S are influent and effluent soluble substrates respectively (mg/L), P_0 is the total SMP concentration if no SMP degradation occurs (mg/L), Θ_c is sludge retention time (d), X_a is MLVSS (mg/L), $K_1 = 0.18$ $gCOD_{products}/gCOD$, $K_2 = 0.2$ $gCOD_{product}/gVSS.d$, and $K_{3M} = 5$ $gCOD_{products}/gVSS.d$.

K_s, K, Y, and K_d were determined to be 29 ± 2 $mgCODL^{-1}$, 1.6 ± 0.2 $mgCOD(mgVSSd)^{-1}$, 0.47 ± 0.07 mgVSS $mgCOD^{-1}$, and 0.08 ± 0.01 d^{-1} respectively at 11°C. According to the Monod parameters, theoretical cell growth and substrate removal equations are depicted in Figure 3.12. The average percentages of the discrepancy between experimental data and Monod fit for substrate (S) removal and cell (X) growth were 4.9% and 2.2% respectively (Figure 3.12).
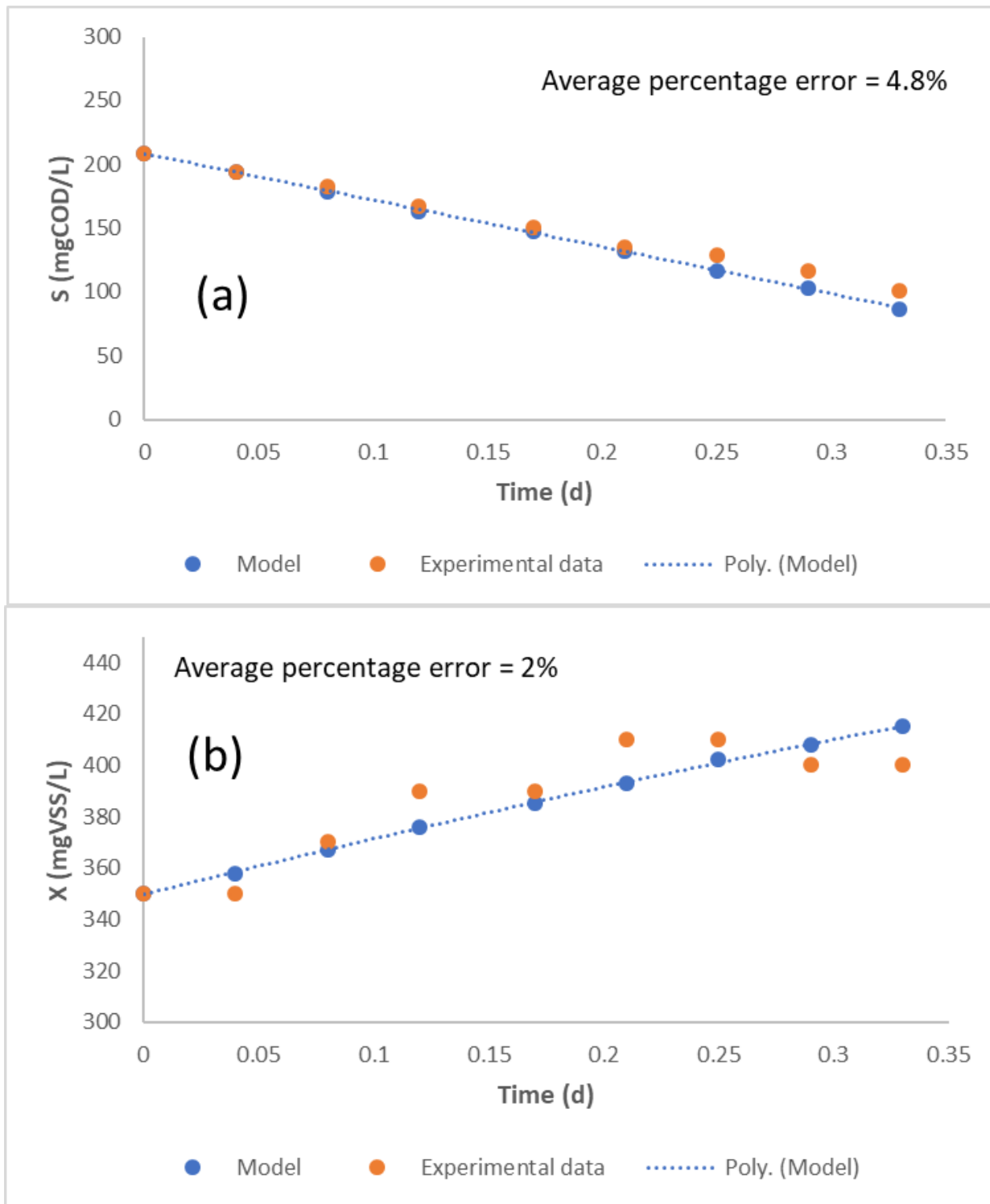
Figure 3.12. Monod fit and experimental data for substrate removal (a) and cell growth (b) at 11°C

The average biomass yields in the PHB at 22°C and 11°C determined from the plot of cumulative VSS produced versus cumulative COD removed which were 0.56 mgVSS mgCOD^{-1} and 0.51 mgVSS mgCOD^{-1} respectively, consistent with the 0.47 mgVSS mgCOD^{-1} derived from the Monod model.

Table 3.7 summarizes the PPB kinetic parameters simulated in MATLAB. By comparison with activated sludge ASM2 (Henze et al.,1995) parameters, it appears that while K_s of PPB of 20-29 mgCODL^{-1} appears to be in the range of the 10-60 mgCODL^{-1} for activated sludge, both K and μ_{max} for the anaerobic PPB are significantly lower than for the activated sludge. Using the abovementioned PHB yields, the estimated substrate concentration ranges from 16 to 35 mgCODL^{-1} corresponding to 10 to 22 mgBOD$_5$L^{-1}. By comparison, the PPB kinetics reported by Puyol et al. (2017) would result in effluent BOD$_5$ concentration of 5 mgL^{-1}. It should be noted however, that the biomass yield of 0.63 gVSS gCOD^{-1} and the COD of PPB of 1.78 mgCOD mgVSS^{-1} reported by Puyol et al. (2017) would translate to 1.12 mgCOD$_{biomass}$ mgCOD$_{subs}$, which is atypically high.

Table 3.7. Summary of PPB kinetics parameters (Municipal wastewater)

Bacteria	Temperature (°C)	Ks (mgCOD/L)	K (mgCOD/mgVSSd)	Y (mgVSS/mgCOD)	μ_{max} (1/d)	Kd (1/d)	References
PPB	22	20	1.9	0.47	0.89	0.07	This study
PPB	11	29	1.6	0.47	0.75	0.08	
PPB	22	20	2.4	0.63	1.54	0.09	Puyol et al., 2017

3.4. Conclusions

The application of purple phototrophic bacteria in a photobioreactor at 22°C and 11°C offers the possibility of achieving high simultaneous removal and recovery of COD, N, and P by PPB in both moderate and cold climate regions. The results of this research are summarized as follows:

1. PPB performance at 11°C was comparable to 22°C, achieving high COD, N, and P removal efficiencies from municipal wastewater.

2. The optimum HRT for COD, N and P removal was 9 hours at both temperatures.

3. High phosphorous removal, without the addition of an external carbon source was achieved with an average effluent concentration of $1.3 mgL^{-1}$. Thus, PPB can be used with chemical polishing to achieve $<1 mgTPL^{-1}$.

4. Monod Kinetic parameters K_s, K, Y, and K_d were determined to be 20-29 $mgCODL^{-1}$, 1.6-1.9 $mgCOD(mgVSS.d)^{-1}$, 0.47 $mgVSS mgCOD^{-1}$, and 0.07-0.08 d^{-1} at temperatures 11°C-22°C.

5. Based on Monod kinetics, it appears that the effluent soluble BOD_5 are 10-22 mgL^{-1} which are higher than the estimated BOD_5 of less than 10 mgL^{-1} reported in the literature.

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Chapter 4

The effect of low light intensity on purple phototrophic bacteria (PPB) performance for municipal wastewater treatment[1]

4.1. Introduction

PPB have a high potential in wastewater treatment due to the removal of chemical oxygen demand (COD) and ammonia through assimilation (Azad et al., 2001; Takabatake et al., 2000), phosphorous uptake by polyphosphate (polyP) formation (Hiraishi et al., 1991), as well as nitrate removal through denitrification (Kim et al., 2004). In order to prove the capability of the purple phototrophic bacteria (PPB) for simultaneous removal of chemical oxygen demand (COD), nitrogen (N), and phosphorous (P) from municipal wastewater, Hulsen et al. (2016) operated a long-term laboratory scale photobioreactor at infrared (IR) light intensity of 50 Wm^{-2}. It was also suggested that the photobioreactor's (PHB) operation at IR intensity of 20 Wm^{-2} is possible, which would considerably reduce the PPB operational costs (Hulsen et al., 2017).

Only a few studies using defined PPB culture and artificial wastewater were carried out in batch tests to evaluate the PPB performance at low light intensity (< 20 Wm^{-2}) (Kim et al., 2004; Zhou et al., 2014; Prasetsan et al., 1993). However, a continuous-flow photobioreactor using mixed PPB culture and real wastewater must be used in order to evaluate the effect of the low light intensity on PPB performance in wastewater treatment. A series of the batch tests were conducted by Kim et al. (2004), to evaluate the organic acid removal by a PPB strain (*Rhodopseudomonas palustris*) at different light intensities of 8-40 Wm^{-2} to achieve the optimum light intensity. The optimum light intensity was at 4 klux (32 Wm^{-2}) for the removal of organic acids (acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, and caproic acid) by *Rhodopseudomonas palustris*.

Zhou et al. (2014) demonstrated that the optimum light intensity for PPB biomass production and COD removal is 2 klux (16 Wm^{-2}) using a series of batch tests at different light intensities of 0.5, 1, 2, 4, and 8 klux which equate to 4, 8, 16, 32, and 64 Wm^{-2}. It was noted that the COD removal efficiency decreased with the higher light intensity than 5 klux (40 Wm^{-2}) which was due to PPB's light inhibition (Zhang et al., 2014; Qu et al., 2011). Prasetsan et al. (1993) indicated that the optimum light intensity for the growth of *Rhodocyclus gelatinosus* (PPB stain) and COD removal from diluted tuna condensate wastewater was 24 Wm^{-2} using batch cultivation tests at 30°C.

The amount of bacteriochlorophyll and carotenoid pigments produced by purple phototrophic bacteria (PPB) directly affects the PPB's obtained energy and its growth (Kue et al., 2012). The quantity of these pigments varies based on different light intensities. Holt and Marr, (1965) demonstrated that *Rhodospirilllum rubrum* grown at low infrared (IR) light contained more bacteriochlorophyll than PPB grown at high light intensity. PPB can adapt to low light intensity by an increase in the bacteriochlorophyll content. Cohen-Bazire et al. (1957) found that in *Rhodospirillum rubrum* and *Rhodopseudomonas spherioides* (PPB strains), the rate of bacteriochlorophyll synthesis is inversely linked to the light intensity. When the light intensity is altered, the pigments adjust their populations by a transient stimulation or suppression. A decrease in light intensity causes an acceleration in the rate of bacteriochlorophyll pigment synthesis; inversely, an increase in the light intensity causes a suppression in the rate of pigment synthesis.

The previous studies offered PPB as a technology to treat wastewater with high COD, N, and P removal efficiencies and simultaneous PPB biomass production with high protein content and high nutrient recovery (Hulsen et al., 2016; Hulsen et al.,2017). Using artificial lights with high intensity challenges the economic feasibility of the PPB technology, hence, light intensity optimization is crucial to enable this technology. The objectives of the current study are; (a) assessment of PPB performance at IR intensities of as low as 3 Wm^{-2} and 1.4 Wm^{-2} (b) PPB enrichment and simultaneous substrate removal to below target limits of 50 $mgCODL^{-1}$, 10 $mgNL^{-1}$, and 1 $mgPL^{-1}$ (c) determination of the optimum hydraulic retention time (HRT) at IR light intensities of 3 and 1.4 Wm^{-2} (d) determination of the Monod kinetic parameters at 3 Wm^{-2}, at HRT, and SRT of 9 hours and 5 days respectively (e) the effect of light-dark cycles of 2 h-2 h on the PPB performance at IR light intensity of 1.4 Wm^{-2}.

4.2. Materials and methods

4.2.1.   Raw wastewater

Municipal wastewater, for this experiment was collected from the Greenway treatment plant located in London, Ontario. After collection, the wastewater was immediately stored in a cold room at a temperature of 4°C. The wastewater was allowed to settle for a period of 2 hours prior to being used as the feed for the photobioreactor. The primary effluent municipal wastewater characteristics are summarized in Table 4.1.

Table 4.1. Municipal wastewater characteristics

Parameters	Values
TCOD (mg/L)	430 ± 50 (n=30)
SCOD (mg/L)	210 ± 45 (n=30)
TSS (mg/L)	160 ± 20 (n=20)
VSS (mg/L)	100 ± 15 (n=20)
TN (mg/L)	43 ± 5 (n=30)
TKN (mg/L)	42 ± 5 (n=30)
NH_4-N (mg/L)	32 ± 4 (n=30)
NOx(mg/L)	0.95 ± 0.1 (n=30)
TP (mg/L)	6.5 ± 0.9 (n=30)
SP (mg/L)	3.7 ± 0.5 (n=30)

4.2.2.   PPB enrichment

A series of fed-batch tests were carried out to enrich the PPB in municipal wastewater under low IR light intensity of 3 Wm^{-2}. Flat foam sheets were used to set the IR light intensity to 3 Wm^{-2} (Appendix B). Raw municipal wastewater was anaerobically illuminated while ethanol was dosed at 100 mgCOD/L. The influent SCOD, NH_4, and PO_4 concentrations were 396 mg/L, 30 mg/L, and 3.4 mg/L respectively. The batch tests were conducted using two bottles with a working

volume of 250 mL. The bottles were continuously mixed at 100 rpm. The samples were allowed to be settled for the duration of 2 hours on days 4, 8, 12 16, and 20, 200 mL of the supernatant were discarded and replaced with 200 mL fresh raw wastewater that were added to the 50 mL PPB inoculum which remained in the bottles after the settlement period. The SCOD, NH_4, and PO_4 concentrations were measured every 4 days to evaluate the PPB growth and COD, N, and P removal from the municipal wastewater.

4.2.3. Anaerobic membrane photobioreactor configuration at low infrared (IR) intensity

The 2 L acrylic rectangular photobioreactor (PHB) with a submerged flat sheet membrane (Kubota, Osaka, Japan) with an area of 0.12 m^2 depicted in Figure 4.1 was used for this study. The length, width and height of the PHB were 34, 2 and 40 cm respectively. Four infrared LED lamps (96 IR LED for Night Vision Camera) using foam sheets as diffusers were used to illuminate the photobioreactor at 3, and 1.4 Wm^{-2}. IR light intensity was measured using IR sensor (Ocean Optics HR 4000). A vacuum pump (Cole Parmer, Air Cadet, R-K 07530-85) aids in continuously mixing the photobioreactor with internal gas from its headspace. A diffuser at the bottom of the photobioreactor transports the gas through the vacuum pump to the photobioreactor. Raw wastewater (primary effluent) was continuously added to the photobioreactor with the aid of a peristaltic pump (Cole Parmer, 7520-67 Masterflex Console Drive Peristaltic pump). The PHB was operated at flow rates of 1.3, 2.6, and 5.2 Ld^{-1}, corresponding to HRTs of 36, 18, and 9h respectively.

Figure 4.1. Continuous membrane photobioreactor configuration at 3 Wm⁻² (left), 1.4 Wm⁻² (center) and light-dark cycles of 2 h-2 h at light intensity of 1.4 Wm⁻² (right)

Experimental analysis was conducted once every three days for both the reactor influent and effluent and the sludge was withdrawn from the bottom of the photobioreactor to control sludge retention time (SRT). During the entire experimentation phase (7 periods) the photobioreactor's average temperature and pH were respectively 22°C and 7.3. Steady-state mass balances were calculated according to the equation1 at an SRT of five days to determine the COD, nitrogen and phosphorus mass closures.

$$\text{Mass closure (\%)} = \frac{\text{Effluent conc (mgL}^{-1}) \,.\, \text{Effluent volume (Ld}^{-1}) + \text{Sludge conc (mgL}^{-1}) \,.\, \text{Sludge volume (Ld}^{-1})}{\text{Influent conc (mgL}^{-1}) \,.\, \text{Influent volume (Ld}^{-1})} \qquad (1)$$

The summary of the operating conditions is depicted in Table 4.2. The photobioreactor was started using a 200 mL PPB inoculum from the fed-batch tests conducted at IR light intensity of 3 Wm⁻² (Appendix B). During start-up (18 days), the PHB was operated at the HRT of 36 hours with no biomass withdrawal. During the steady state, the photobioreactor (PHB) was operated at different HRTs of 36 h, 18 h and 9 h. The SRT was set to 5 days during periods 1-7 when the PHB was illuminated at 3 and 1.4 Wm⁻². In period 7, the PHB was operated in the light-dark cycles of 2 h-2 h under IR light intensity of 1.4 Wm⁻² at the SRT of 5 days. The PHB was opened two times, at days 24 and 60 after periods 2 and 4, respectively to remove the biofilm from the membrane and the reactor's walls. The PHB's operation for long periods at the short HRT of 9 h led to the formation of the PPB biofilm which impaired the performance of the photobioreactor.

Table 4.2. The summary of PHB operating conditions

Period	HRT (h)	SRT (d)	Ethanol (mg/L)	IR light intensity (Wm^{-2})
Startup	36	-	200	3
Period 1 Day 0-12	36	5	–	3
Period 2 Day 13-24	36	5	200	3
Period 3 Day 25-42	18	5	200	3
Period 4 Day 43-60	9	5	300	3
Period 5 Day 61-72	18	5	300	1.4
Period 6 Day 73-90	9	5	300	1.4
Period 7 Day 91-110	9	5	300	1.4 (light-dark cycles of 2h-2h

4.2.4.   Analytical methods

The following parameters were measured based on standard methods for examination of water and wastewater 18th edition (APHA AWWA and WEF, 1992). Total chemical oxygen demand (TCOD), total nitrogen (TN), total kjeldahl nitrogen (TKN), total phosphorous (TP) and soluble chemical oxygen demand (SCOD) were determined according to the HACH method (HACH Odyssey DR 2800). Total suspended solids (TSS) and volatile suspended solids (VSS) were determined according to standard methods (APHA, 1998). All soluble samples were specified using a membrane filter with a 0.45 µm pore size (GN Metricel Membrane Disc Filters, Pall Laboratory).

4.2.5.   Microbial growth

The optical density (OD) test was used to evaluate the absorbance behaviour of the samples taken from the photobioreactor during periods 3, and 5 at an HRT of 18 h and light intensities of 3, and 1.4 Wm^{-2} respectively. This analysis performed using DR 3900 spectrophotometer demonstrated the presence of bacteriochlorophyll and carotenoid pigments in the PPB's cell. The optical density (OD) peaks at wavelengths of 590, 800, and 860 nm which show the presence of bacteriochlorophyll pigments, while, the two peaks at 460 and 488 nm reflect the presence of carotenoid pigments in the PPB.

4.2.6.   Monod kinetic model

The Monod Kinetic simulation was carried out in MATLAB (MATLAB R2017a, The MathWorks Inc., Natick, MA) to determine the parameters K_s, K, K_d and μ_{max}. The nonlinear curve fitting command (lsqcurvefit) was employed to establish the best fit of the experimental data X (t) and S (t) from the batch tests to Monod kinetic parameters. The batch tests were carried out using a combination of 150 mL fresh wastewater, 100 mgCODL^{-1} ethanol and 50 mL PPB inoculum from the photobioreactor at an HRT of 18 h and an IR intensity of 3.3 Wm^{-2}. The mixture of the inoculum, fresh wastewater and ethanol had initial SCOD, NH$_4$, PO$_4$, and VSS concentrations of 292, 24.5, 3, and 360 mgL^{-1} respectively. 10 mL samples were collected hourly for a period of eight hours to measure the soluble COD and VSS concentrations.

4.3. Results and discussion

4.3.1.   IR light intensity measurement

The profile of the illumination intensity-wavelength is depicted below in Figure 4.2. The IR light intensity was determined using integration of irradiance curve between the wavelengths of 750 nm and 900 nm to calculate the area under the curve which demonstrates the light intensity.
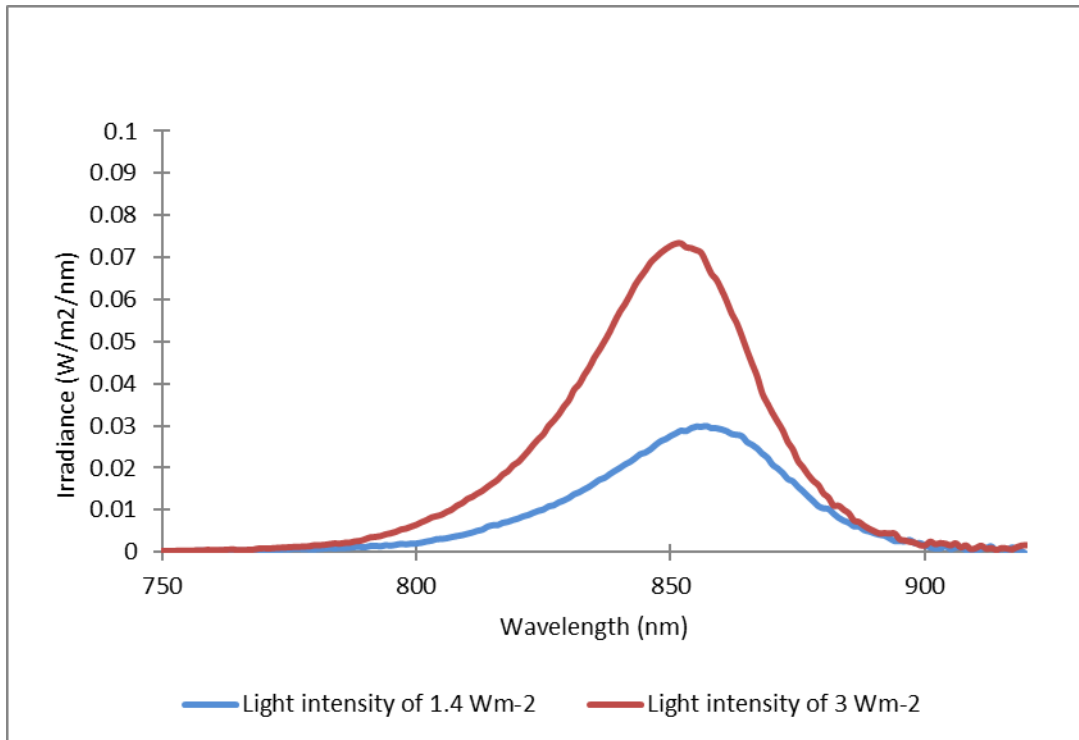
Figure 4.2. Irradiance-wavelength profile at 3, and 1.4 Wm^{-2}

4.3.2. PPB fed-batch tests

At day 20, the SCOD, NH$_4$, and PO$_4$ concentrations were 134, 13, and 1.2 mgL^{-1} respectively, corresponding to the SCOD, NH$_4$, and PO$_4$ removal efficiencies of 66%, 57%, and 65%. The low SCOD, NH$_4$, and PO$_4$ removal efficiencies at day 8 were 12%, 9%, and 15% respectively, which was due to the inadequate PPB culture at the beginning of the fed-batch tests (days 1-8).
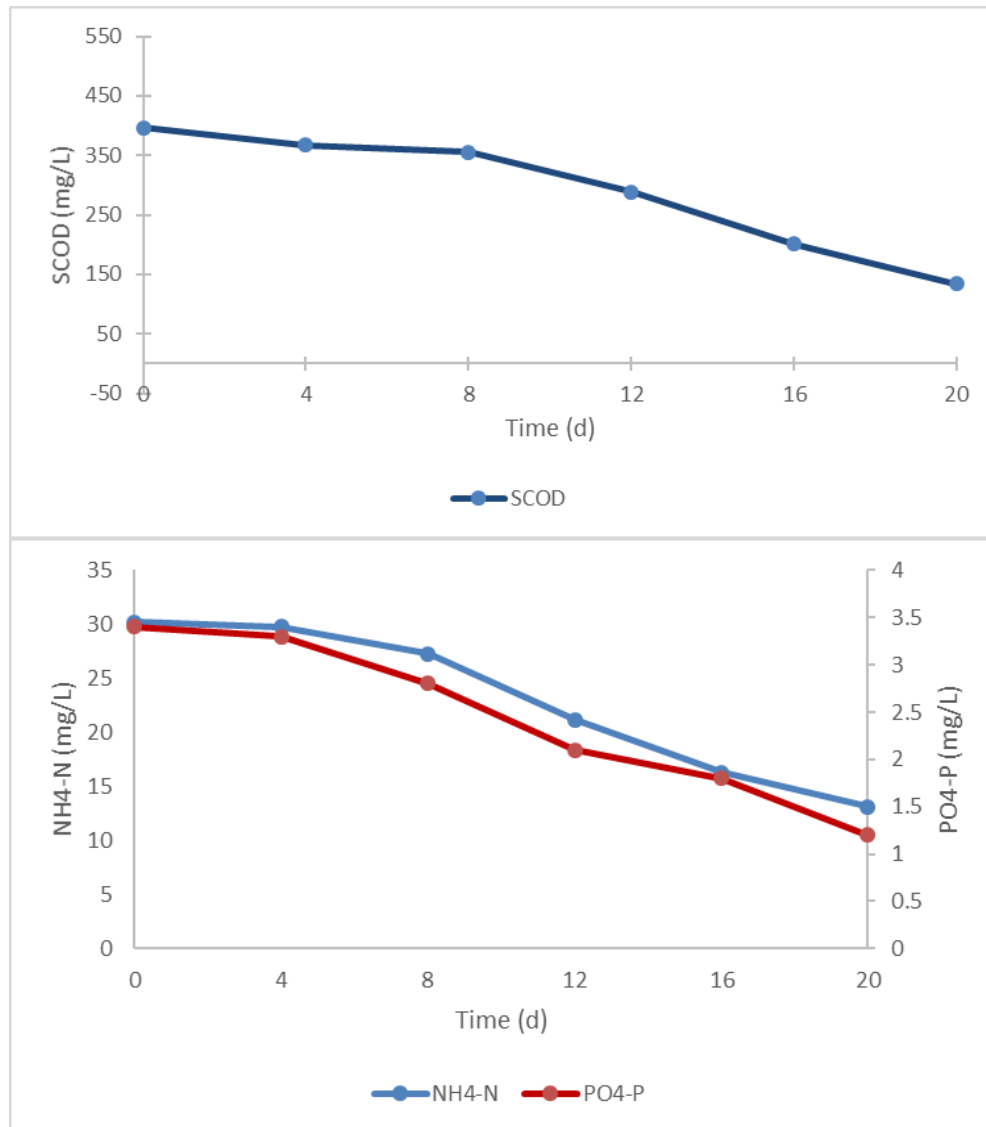
Figure 4.3. COD, N, and P concentrations in the fed-batch tests

### 4.3.3.   PPB performance at low light intensity

During the start-up, the average effluent COD, N, and P concentrations were 137 ± 82 mgL^{-1}, 15 ± 6 mgL^{-1} and 1.5 ± 1 mgL^{-1} respectively, corresponding to COD, N, and P removal efficiencies of 77%, 62%, and 73% (based on 6 samples). Steady-state effluent TCOD, TN, and TP concentrations (based on 36 samples) as well as TCOD, TN, and TP removal efficiencies are summarized in Table 4.3, while the temporal COD, N, and P variations are illustrated in Figures 4.4, 4.5, and 4.6 respectively. The effluent substrate concentrations were below target limits of 50 mgCODL^{-1}, 10

mgNL^{-1}, and 1 mgPL^{-1} during periods 2, 3, and 4 when ethanol was dosed at the average concentration of 260 mgCODL^{-1}. These results showed PPB's high potential for municipal wastewater treatment at IR light intensity as low as 1.4 Wm^{-2}. In periods 1-6, the average TCOD: TN: TP uptake ratio by PPB at steady-state was 100: 6: 1; however, this ratio decreased to 100: 4.5: 0.59 during period 7 when the PHB was operated in light-dark cycles of 2 h- 2 h. The PHB color changed from red to purple in period 7 indicating that the PPB culture in light-dark cycles of 2 h- 2 h was different than the PPB culture in periods 1-6 (full-light). Steady state mass balances in periods 4 and 6 when the PHB was operated at the HRT of 9 h, showed COD mass closure of 82%±4% and 81%±3%, TN mass closure of 86%±4% and 83%±2% as well as TP mass closure of 83%±4% and 81%±1% respectively.

Table 4.3. Steady-state photobioreactor performance for different periods

Period	TCOD removal (%)	Effluent TCOD (mg/L)	TN removal (%)	Effluent TN (mg/L)	TP removal (%)	Effluent TP (mg/L)
1	92 ± 0.8	36 ± 3	44 ± 3	24 ± 2	77 ± 3	1.34 ± 0.2
2	95 ± 0.3	34 ± 3	85 ± 2	6 ± 1	87 ± 3	0.72 ± 0.1
3	95 ± 0.4	34 ± 3	86 ± 3	6 ± 2	93 ± 1	0.4 ± 0.1
4	94 ± 0.5	40 ± 2	80 ± 1	8 ± 1	90 ± 2	0.54 ± 0.1
5	95 ± 0.3	39 ± 2	85 ± 2	5 ± 1	91 ± 1	0.66 ± 0.07
6	94 ± 0.5	40 ± 3	81 ± 2	7 ± 1	88 ± 1	0.9 ± 0.08
7	94 ± 0.7	43 ± 3	73 ± 3	11 ± 2	69 ± 4	1.8 ± 0.3
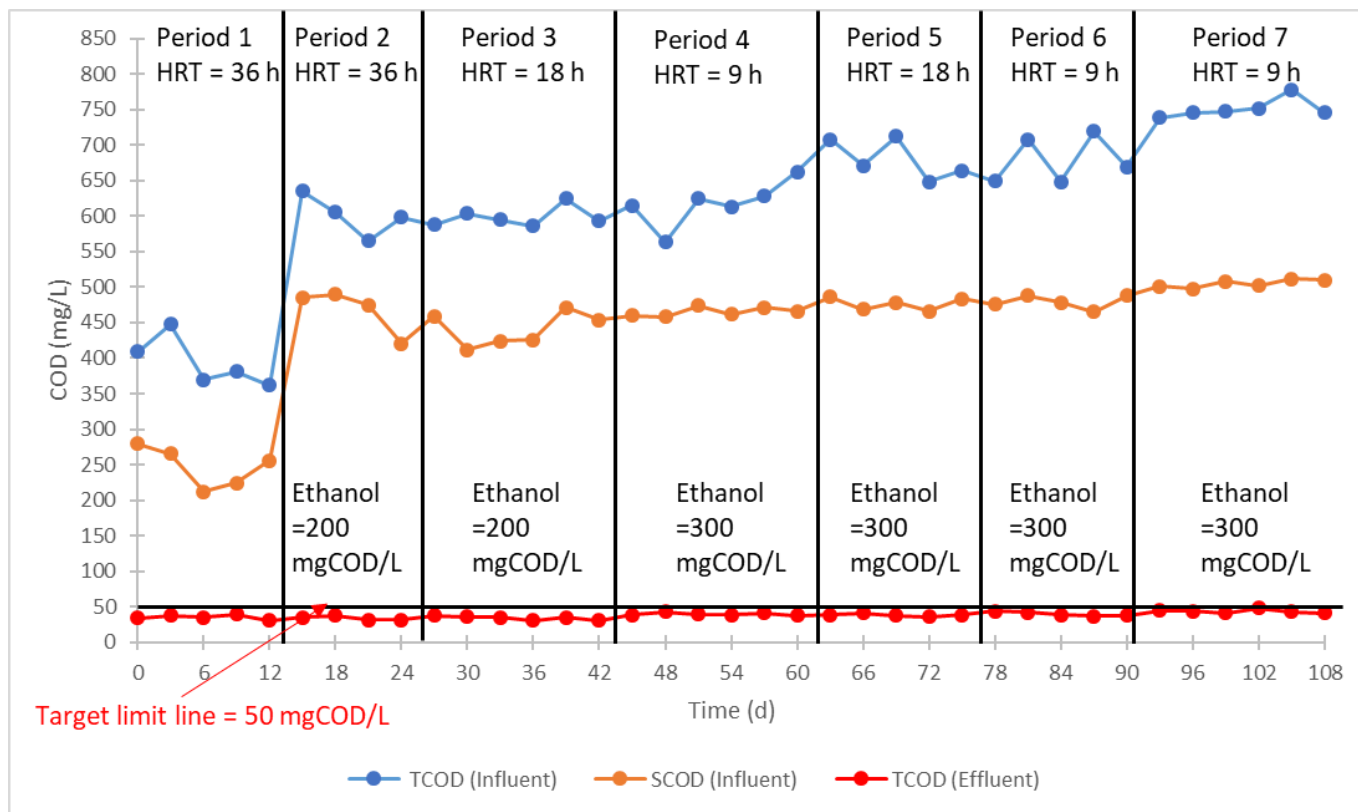
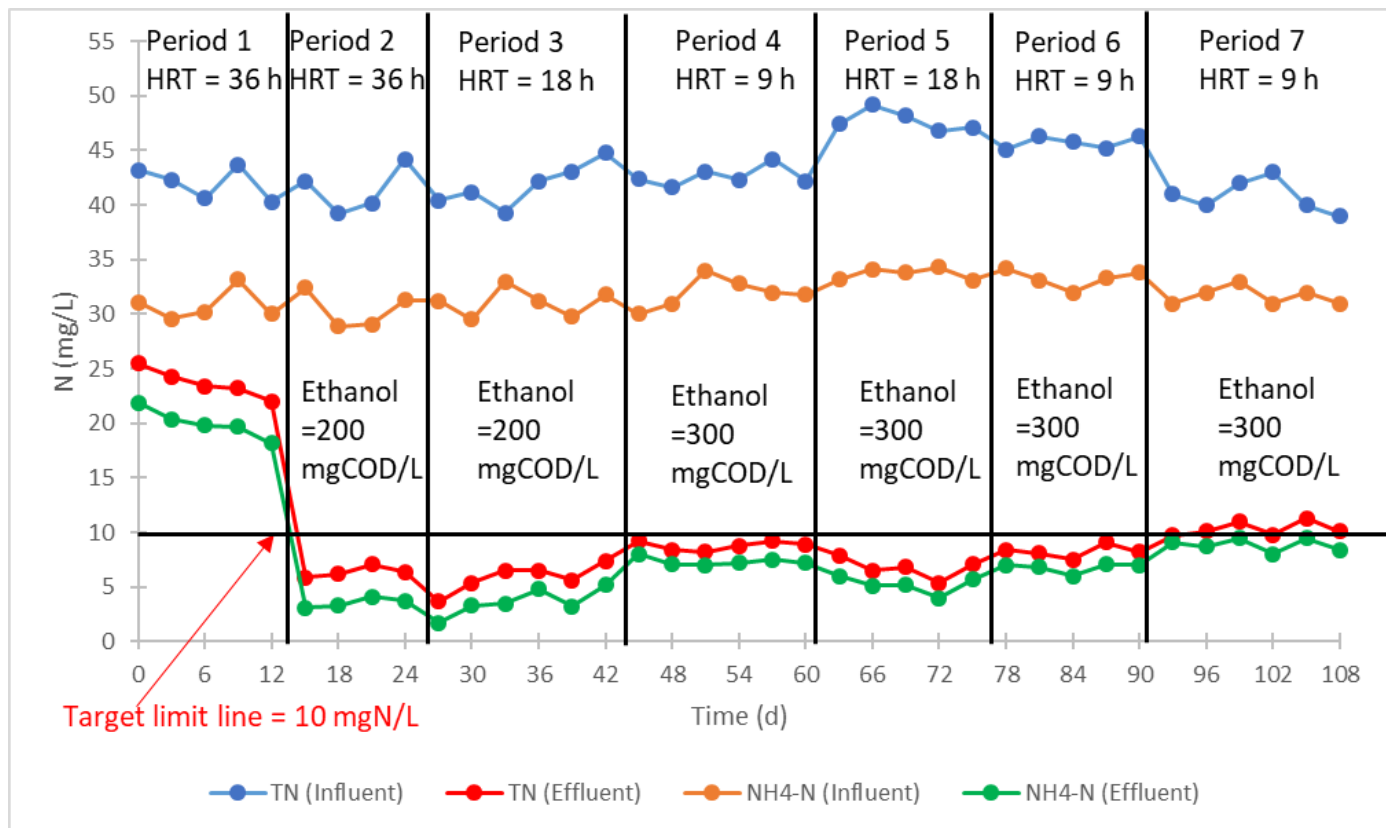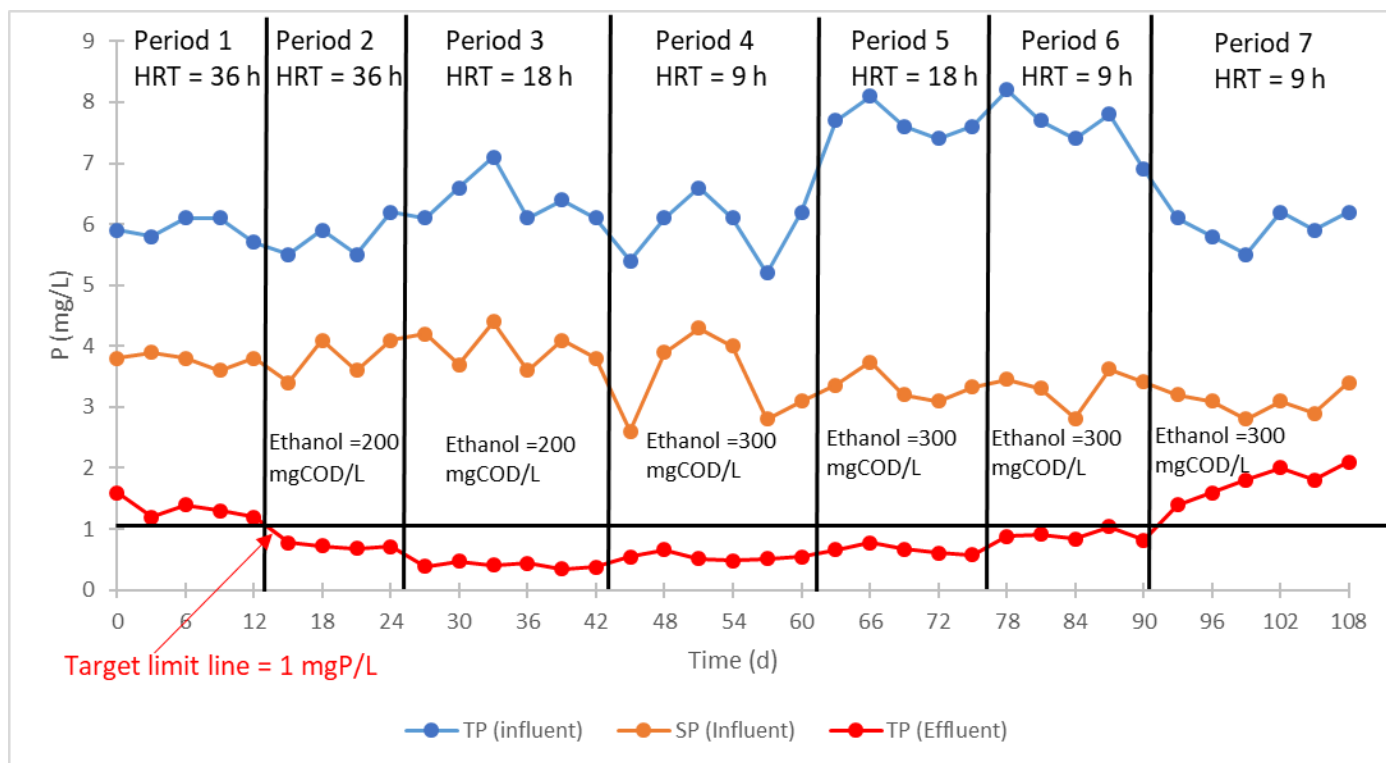**Figure 4.4. Influent and effluent COD concentrations at 3 and 1.4 Wm⁻²**

Figure 4.5. Influent and effluent N concentrations at 3 and 1.4 Wm⁻²

Figure 4.6. Influent and effluent P concentrations at 3 and 1.4 Wm^{-2}

In periods 2-6, the average effluent COD, N and P concentrations were 37 mg/L, 6 mg/L and 0.62 mg/L respectively. The high effluent nitrogen concentration of 24 mgL^{-1} (removal efficiency of 44%) in period 1 was due to the inadequate carbon source. The nitrogen removal efficiency increased significantly to more than 80%) with the addition of ethanol in periods 2-6. The PHB operation at different HRTs (9-36 h) had no substantial effect on TCOD removal efficiencies (> 92%). Effluent N, and P concentrations increased in both periods 4, and 6 when HRT decreased from 18 h to 9 h. The average COD/N/P uptake ratio by PPB was 100/6±0.2/1±0.1 (periods1-6). The temporal variation of the above-mentioned ratio in periods 1-6 is depicted in Figure 4.4. It is very evident from Figure 4.4, that the ratio of COD/N/P uptake by the PPB was stable throughout the study irrespective of HRT and light intensity. The impact of light intensity of 1.4 Wm^{-2} in periods 5, and 6 was not detrimental to the PPB performance in comparison with periods 3-4 when the PHB was operated under IR light intensity of 3 Wm^{-2}.

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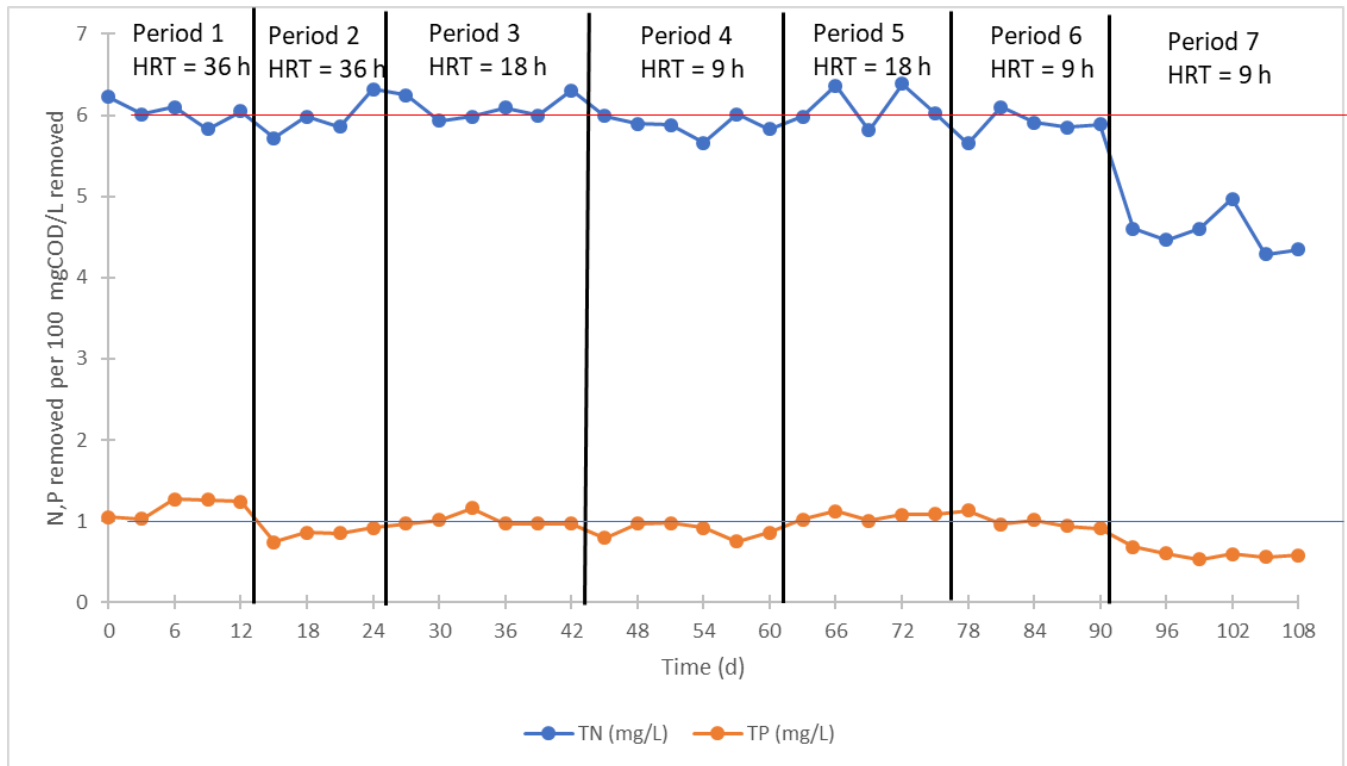
Figure 4.7. TN, and TP removed per 100 mgCOD/L removed (lines at 1, and 6 mg/L represent the average P and N ratio removed by PPB per 100 mgCOD/L in periods 1-6)

In period 7, when the PHB was operated in the light-dark cycles of 2 h- 2 h, the average effluent nitrogen and phosphorous concentrations were 11 and 1.8 mg/L respectively; however, the average effluent COD concentration was 43 mg/L. The high effluent phosphorous concentration in this period was due to the release of phosphorous from the PPB biofilm formed on the membrane. Table 4.4 shows a comparison of the SCOD, NH_4, and SP concentrations in the PHB and the permeate (final effluent). The average soluble phosphorous concentration in the PHB was 0.49 ± 0.2 mg/L, which was significantly lower than the effluent phosphorous concentration of the samples taken from the final effluent (1.8 ± 0.3 mg/L). The SCOD, NH_4, and SP analysis of the samples taken from the final effluent and from the PHB at days 103-108 indicated that 1.4 mg/L soluble phosphorous was released from the PPB biofilm on the membrane, which significantly decreased the PPB performance for phosphorous removal in period 7. Based on the Table 4.4, the average effluent SCOD and NH_4 concentrations were 42 and 9 mg/L respectively; however, the SCOD and NH_4 concentrations of the filtered samples taken from the PHB were 55 and 10 mg/L respectively. A paired t-test was conducted to compare SCOD, NH_4, and SP concentrations in the PHB and the permeate (final effluent) to evaluate the effect of the membrane on the SCOD, NH_4 and SP removal

efficiencies and the PPB performance. Based on the results of the t-test, there were significant differences in the SCOD, NH_4, and SP concentrations between the final effluent and the PHB at the 95% confidence level. The two-tailed P value for SCOD, NH_4 and SP values were 0.0001, 0.0014, and 0.0001 respectively. The SCOD, NH_4 and SP average concentrations and their standard deviation are summarized in Table 4.4.

The simultaneous COD uptake and phosphorous release in this period may imply that the polyphosphate-accumulating organisms (PAOs) accumulated on the membrane during the light-dark cycles of 2 h-2 h, particularly that the ratio of COD uptake to P release was 9.3:1, close to typical for PAOs.

Table 4.4. The effect of the PPB biofilm on the PPB performance

Time (d)	Filtered samples taken from the PHB			Samples taken from effluent pump through the membrane		
	SCOD (mg/L)	NH_4 (mg/L)	SP (mg/L)	SCOD (mg/L)	NH_4 (mg/L)	SP (mg/L)
103	58	10.4	0.2	41	9.1	1.8
104	56	9.8	0.4	40	8.7	1.6
105	51	10.1	0.9	42	9.5	1.8
106	60	9.8	0.6	43	9	2
107	55	10.4	0.5	44	9.5	1.9
108	51	9.6	0.6	39	8.4	2.1
Average	55	10	0.5	42	9	1.9
Standard deviation	4	0.3	0.2	2	0.4	0.2

The PHB's MLSS and MLVSS during periods 1-7 are depicted in Figure 4.5. The ranges of MLSS and MLVSS in periods 1-7 varied from 290 to 1250 mgL^{-1}, and from 240 to 1090 mgL^{-1} respectively. It is obvious from Figure 4.8, that the variations of the MLSS, and MLVSS were correlated with the HRT. The average ratio of MLVSS to MLSS was 0.85±0.05 which was stable during periods 1-7. The average biomass yield of 0.50±0.02 $mgVSS\ mgCOD^{-1}$ in periods 3-6 demonstrates that the PPB performance remained the same when light intensity decreased from 3 to 1.4 Wm^{-2}.
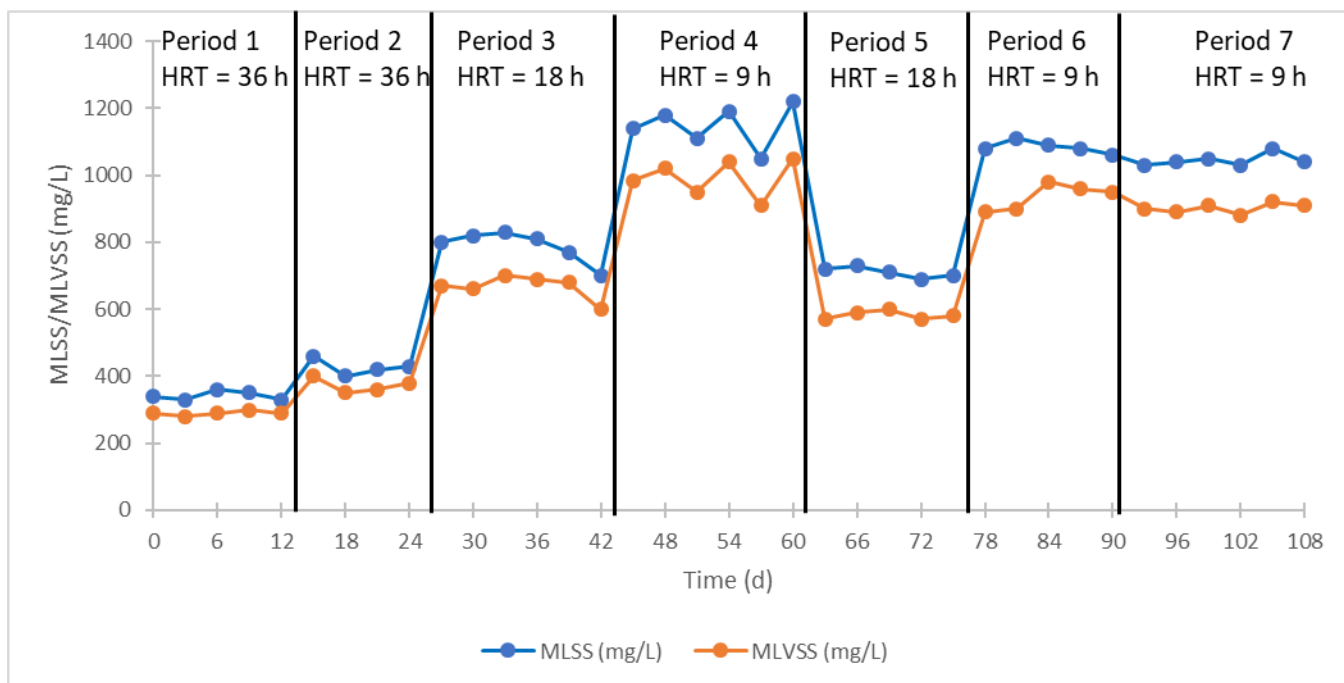
Figure 4.8. PHB's MLSS and MLVSS in periods1-7

4.3.4. Microbial growth

The results of the optical density (OD) tests at light intensities of 3, and 1.4 Wm^{-2} as well as the light- dark cycles of 2 h-2 h at the IR light intensity of 1.4 Wm^{-2} are depicted in Figure 4.6. The OD tests were carried out when the PHB was operated at the HRT and the SRT of 18 hours and 5 days respectively in periods 3 and 5. The OD tests were also conducted at the HRT of 9 hours and the SRT of 5 days in period 7 in the light- dark cycles. The OD profile of the samples taken from the photobioreactor at the above-mentioned light intensities showed the presence of bacteriochlorophyll pigments with three peaks at 590, 800, and 860 nm and the presence of carotenoid pigments with two peaks at 460, and 488 nm. These results are consistent with PPB's absorbance trend which shows a similar trend with peaks attributed to the above-mentioned pigments (Cohen-Bazire et al., 1957). According to the Figure 4.5 (a), OD values for the sample grown at 1.4 Wm^{-2} were 4.04, 4.1, 3.5 and 3.94 respectively at the wavelengths of 460,488, 800 and 860 nm. However, OD results for PHB's sample grown at 3 Wm^{-2} were determined to be 4.31, 4.31, 3.05 and 3.35 at the wavelength of 460, 488, 800, and 860 nm respectively. The relatively

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higher OD values at 800, and 860 nm for PPB grown under light intensity of 1.4 Wm^{-2} showed a higher production of the bacteriochlorophyll pigments due to the adaptation of the PPB to low light intensities. However, the higher OD values at 460, and 488 nm for the PPB grown under light intensity of 3 Wm^{-2} indicated that the amount of carotenoid pigments increased with higher light intensities which is consistent with the literature (Zhou et al., 2014).
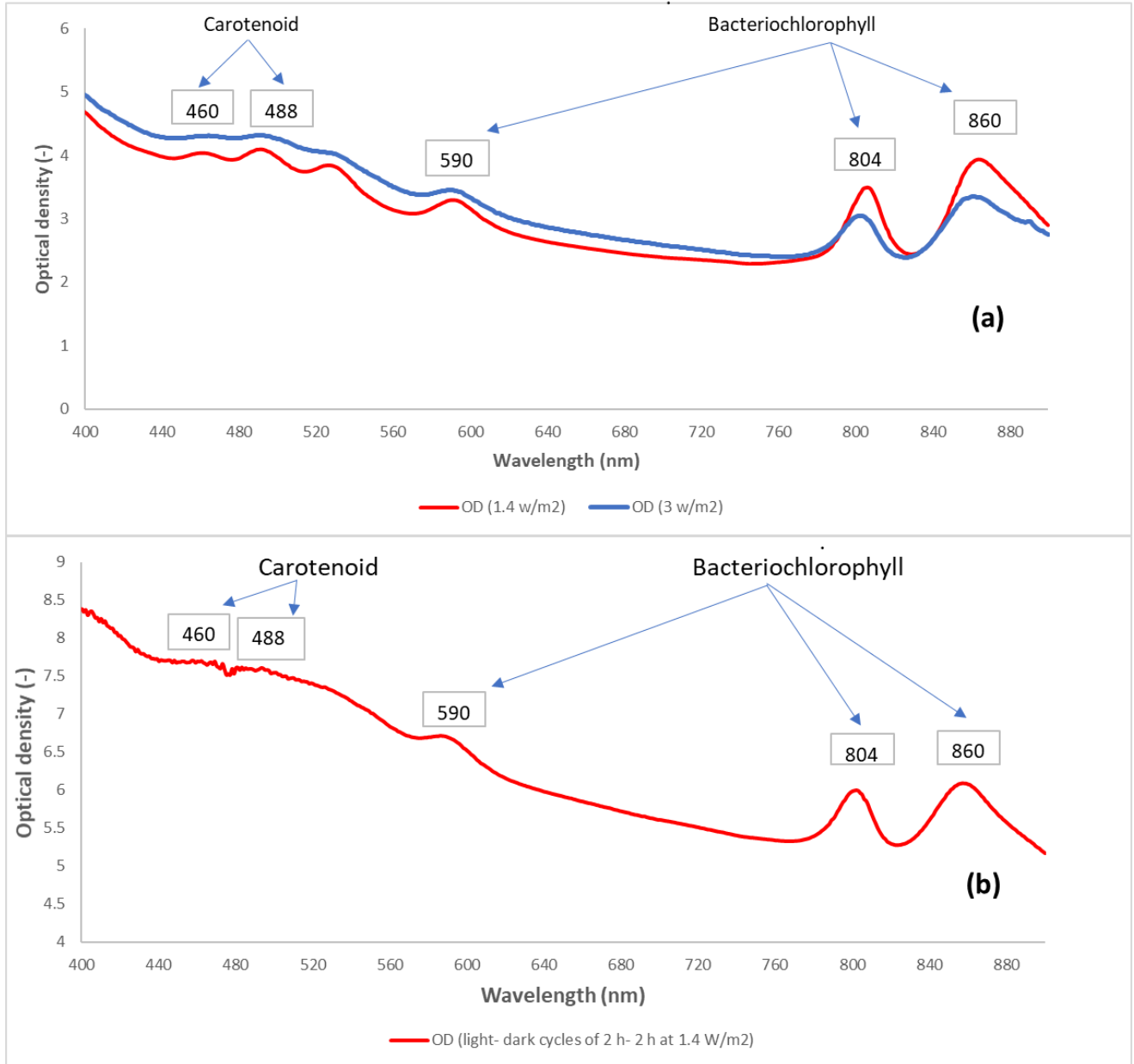

Figure 4.9. Full-spectrum optical density measurement at 3 Wm^{-2} and 1.4 Wm^{-2} (HRT=18 h) (a), and at light-dark cycles of 2 h- 2 h at 1.4 Wm^{-2} (HRT = 9 h) (b)

4.3.4.   Monod kinetic parameters model

Monod kinetic parameters in the batch tests at low IR light intensity of 3 Wm^{-2} were determined by using nonlinear curve-fitting (lsqcurvefit) of the experimental data (X and S) to the Monod kinetic parameters based on Monod equations using MATLAB (MATLAB R2017a, The MathWorks Inc., Natick, MA). K_s, K, Y, and K_d at the low IR light intensity of 3 Wm^{-2} were determined to be 20±4 $mgCODL^{-1}$, 1.9±0.1 $mgCOD(mgVSSd)^{-1}$, 0.49±0.05 mgVSS $mgCOD^{-1}$, and 0.08±0.01 d^{-1} respectively. According to the derived Monod parameters, theoretical cell growth and substrate removal were obtained by solving Monod equations using ODE45. The comparison between the theoretical and experimental cell growth and substrate removal are depicted in Figure 4.7. The average absolute percentages of the discrepancy between experimental data and Monod fit for substrate (S) removal and cell (X) growth were 4% and 3% respectively, which demonstrates the accuracy of the model parameters.
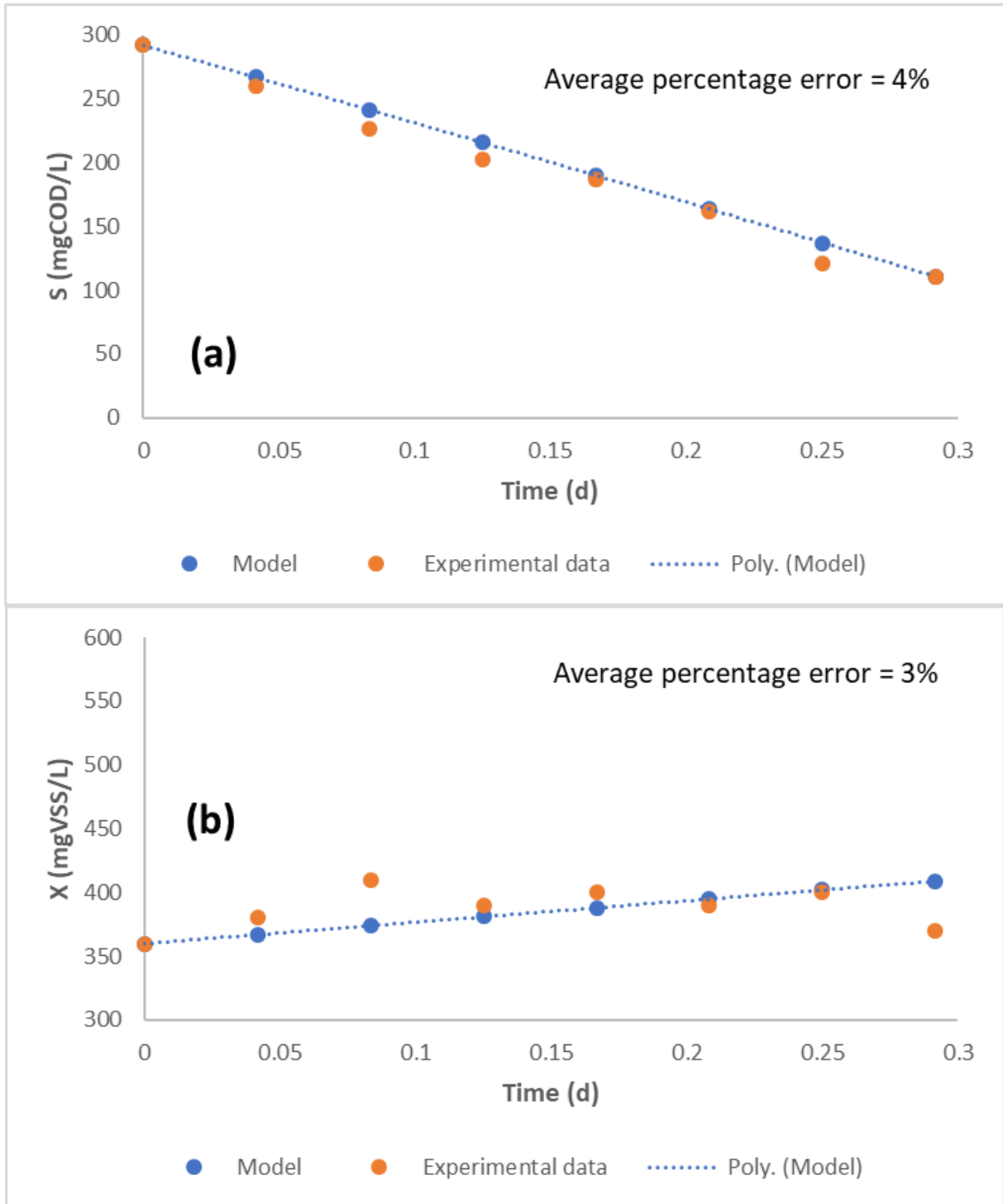
Figure 4.10. Monod fit and experimental data for substrate removal (a) and cell growth (b) at the low intensity of 3 Wm⁻²

According to equation 2, the effluent substrate concentration in a continuous-flow reactor is the function of SRT, Ks, K_d, K and Y. The PHB effluent substrate concentration based on the above-mentioned kinetic parameters and equation 2 was determined to be 8 mgCODL^{-1}, while experimental results showed the average effluent of 37 mgCODL^{-1} which shows that the concentration of the inert soluble COD in the wastewater collected from the Greenway treatment plant is 29 mgCODL^{-1}. This is higher than the inert soluble COD concentration of 14 mgL^{-1} observed by Gupta et al. (2018) in the same wastewater. The soluble microbial products (SMPs) concentration was determined to be 13 mgCODL^{-1} by using equations 3, and 4 (Rittmann et al., 1987). This demonstrated that SMPs were released from microbial product accumulated on the membrane and affected the effluent quality. Base on the above-mentioned results, the COD fractionation of the effluent was: inert soluble COD (16 mgL^{-1}), the soluble microbial products (13 mgCODL^{-1}), and the biodegradable soluble COD (8 mgL^{-1}).

$$S = \frac{K_s \left(1 + K_d \cdot SRT\right)}{SRT \left(\mu_{max} - K_d\right) - 1} \tag{2}$$

$$P = \frac{P_0}{1 + \dfrac{K_{3m} \cdot X_a \cdot \Theta_c}{P_0}} \tag{3}$$

$$P_0 = -K_1 \cdot (S_0 - S) + K_2 \cdot X_a \cdot \Theta_c \tag{4}$$

Where, S_0 and S are influent and effluent soluble substrates respectively (mg/L), X_a is MLVSS (mg/L), Θ_c is sludge retention time (d), P_0 is the total SMP concentration if no SMP degradation occurs (mg/L), $K_1 = 0.18$ gCOD$_{products}$/gCOD, $K_2 = 0.2$ gCOD$_{product}$/gVSS.d, and $K_{3M} = 5$ gCOD/gVSS.d.

Table 4.5 summarizes the PPB Monod kinetic parameters. By comparison with the PPB at high IR light intensity, it appears that PPB performance at the IR intensity of 3 Wm^{-2} was comparable to the intensity of 50 Wm^{-2}.

Table 4.5. The summary of Monod kinetic parameters

Bacteria	Light intensity (Wm^{-2})	Ks $(mgCODL^{-1})$	K $(mgCOD/mgVSSd)$	Y $(mgVSS/mgCOD)$	μ_{max} $(1/d)$	K_d $(1/d)$	References
PPB	3	20±4	1.9±0.1	0.49±0.05	0.93	0.08±0.01	This study
PPB	50	20±4	2.4±0.2	0.63	1.54	0.09±0.02	Puyol et al.(2017)

The main energy inputs for the PHB are lighting and mixing energy for membrane scouring. The mixing energy was determined to be 0.15 kWh/m^3 (Hulsen et al., 2017). Hulsen et al. (2016) utilized high light intensity (50 Wm^{-2}) to evaluate the PPB's viability in municipal wastewater treatment using continuous-flow photobioreactor. The high energy consumption (24 kWh/m^3) for the above-mentioned light intensity challenges the practical application of the PPB technology. Based on the results of this study, high PPB performance was achieved at light intensities of 3, and 1.4 Wm^{-2} which significantly decreased energy consumption for lighting to 1.44, and 0.67 kWh/m^3. These results expand the potential of the PPB technology for municipal wastewater treatment using a membrane photobioreactor, reducing light energy by up to 97%. The comparison of the operating conditions and the performance of the PPB, high rate algal pond (HRAP) and photobioreactors (PBRs) using microalgae are summarized in Table 4.6. Wastewater treatment by PPB offers higher N, and P removal efficiencies at significantly lower HRTs in comparison with HRAP and PBRs using algae. Furthermore, PPB's operational costs are considerably lower than PBRs using microalgae. Large footprint, long HRTs, and moderate N and P removal efficiencies are the drawbacks of HRAP in comparison with PPB.

Table 4.6. The comparison of the operating conditions and the performance of the PPB, high rate algal pond (HRAP) and photobioreactors (PBRs)

Technology	N removal (%)	P removal (%)	HRT (d)	SRT (d)	Light intensity (Wm^{-2})	Energy (mixing) (kWh/m^3)	Footprint (m^2/m^3)	Operational costs	References
HRAP	67	52	2-10[a]	2-10	<100[b] (sunlight)	-	Large	Low	Shoener et al., (2014)
PBR	78	93	2-10[a]	2-10	<100[b]	1.75-3.6	Small	High	Shoener et al., (2014)
PPB	82	90	0.4	3	1.4	0.15[b]	Small	medium	This study

[a] (Jorquera et al., 2010; Larsdotter, 2006)

[b] (Hulsen et al., 2017)

4.4. Summary and Conclusions

The results of this study demonstrate the potential of the purple phototrophic bacteria (PPB) in treating municipal wastewater at IR light intensity of as low as 1.4 Wm^{-2}. These results prove the feasibility of simultaneous non-destructive COD, N, and P removal at low IR light intensity. The results of this study are summarized as follows:

1. Anaerobic phototrophic municipal wastewater treatment with PPB can achieve average effluent COD, N, and P concentrations of below 37 mgL^{-1}, 6 mgL^{-1}, and 0.62 mgL^{-1} with an external COD addition (240 mgL^{-1}) under low IR light intensity of 3, and 1.4 Wm^{-2}.

2. The study demonstrates that the optimized light intensity used for municipal wastewater treatment with PPB is significantly lower than previously indicated in the literature. The energy consumptions attributed to illuminate the PHB at 3, and 1.4 Wm^{-2} were determined to be 1.44, and 0.67 kWh/m^3 respectively which are significantly lower than previous studies (> 24kWh/m^3).

3. The effluent COD, N, and P concentrations throughout period 7 (light-dark cycles of 2 h-2 h) were significantly different than periods 1-6 when the PHB was operated under full-lighting without the accumulation of the PPB biofilm on the membrane. However, the

energy consumption attributed to the lighting decreased significantly, to 0.33 kWh/m^3, using light-dark cycles of 2 h-2 h.

4. The average effluent COD of 37 mgL^{-1} was attributed to the inert COD, SMPs, and biodegradable COD concentrations of 16, 13, and 8 mgL^{-1} respectively.

5. Monod Kinetic parameters K_s, K, Y, and K_d were determined to be 20 mgCODL^{-1}, 1.9 mgCOD (mgVSS.d)$^{-1}$, 0.49 mgVSS mgCOD^{-1}, and 0.08 d^{-1} at low light intensity of 3 Wm^{-2} which are comparable to the Monod kinetic parameters determined at high light intensity from the literature.

4.5. References

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Chapter 5

5.   Conclusions and recommendations

5.1. Conclusions

The detailed findings of this study have been included in chapters 3 and 4. The principal findings of the first study (chapter 3) were as follows:

1. This study demonstrated that the low temperature of 11°C and the short HRT of 9 hours had no detrimental impacts on PPB performance. PPB have a high potential to treat municipal wastewater at 11°C and 22°C with effluent COD, N, and P concentrations below 50 mgL^{-1}, 10 mgL^{-1}, and 1 mgL^{-1} respectively. The addition of an external carbon source (300 mgCODL^{-1}) was required for N, and P removal based on the ratio of COD/N/P uptake by PPB (100/6.1/1.3).

2. The results of the steady-state mass balance indicated high COD, N, and P mass closures (>80%), which is indicative of PPB's substrate and nutrient assimilation.

3. High phosphorus removal was achieved by PPB without the addition of ethanol, although chemical polishing was required to meet the target limit of 1 mgPL^{-1}.

4. Monod kinetic parameters K_s, K, K_d, and Y were 20-29 mgCODL^{-1}, 1.6-1.9 mgCOD (mgVSS.d)$^{-1}$, 0.07-0.08 d^{-1}, and 0.47 mgVSS mgCOD^{-1} respectively at temperatures of 11°C-22°C. Based on Monod kinetic parameters, the effluent soluble BOD$_5$ was estimated to be 10-22 mgL^{-1} which is significantly higher than the value reported in the literature (<10 mgL^{-1})

The major findings of the second study (chapter 4) were as follows:

1. This study demonstrated that the simultaneous non-destructive removal of COD, N, and P from municipal wastewater was feasible using PPB at a light intensity as low as 1.4 Wm^{-2}.

2. High PPB performance was achieved with average effluent COD, N, and P concentrations below 37 mgL^{-1}, 6 mgL^{-1}, and 0.62 mgL^{-1} respectively when ethanol was dosed at 240 mgCODL^{-1}. The results showed that the effluent COD was attributed to the inert COD and

SMPs from the microbial product accumulated on the membrane. The short HRT of 9 h had no detrimental impact on the PPB performance.

3. The energy consumption attributed to the PHB's illumination was determined to be 0.67 kWh/m^3 which is significantly lower than previously indicated in the literature (>24 kWh/m^3).

4. The PPB performance in the light-dark cycles of 2 h-2 h (period 7) was significantly different from its performance during the periods under full-lighting due to the P release and COD uptake by the biofilm on the membrane surface.

5. The low light intensity of 3 Wm^{-2} had no substantial impact on the Monod kinetic parameters K_s, K, K_d, and Y in comparison with the high light intensity of 50 Wm^{-2}.

5.2. Recommendations

The practical application of PPB in municipal wastewater treatment would require further investigation. The recommendations for future work are as follows:

1. Assessment of the PPB performance at IR light intensities of <1.4 Wm^{-2} is recommended to determine the optimum IR light intensity for municipal wastewater treatment by PPB.

2. Different light-dark cycles of 1 h- 3 h and 1 h- 4 h at the IR light intensity of 1.4 Wm^{-2} are recommended to reduce the energy consumption, up to 80% compared to the full-lighting condition.

3. Further investigation of the impact of the biofilm on the PHB performance, particularly on the effluent phosphorous concentration, is required regarding the phosphorous release from the PPB biofilm on the membrane.

4. The addition of an external carbon source is neither financially feasible nor environmentally friendly. Fermentate from PPB biomass digestion (internal carbon source) can be used as an alternative for the external carbon source. Thus, nutrient removal by PPB with an internal carbon source is recommended to optimize the PHB.

5. The application of PPB in lagoon systems is recommended using sunlight with an IR filter which absorbs the spectrums below 790 nm. However, the large footprint may be the drawback of this treatment system.
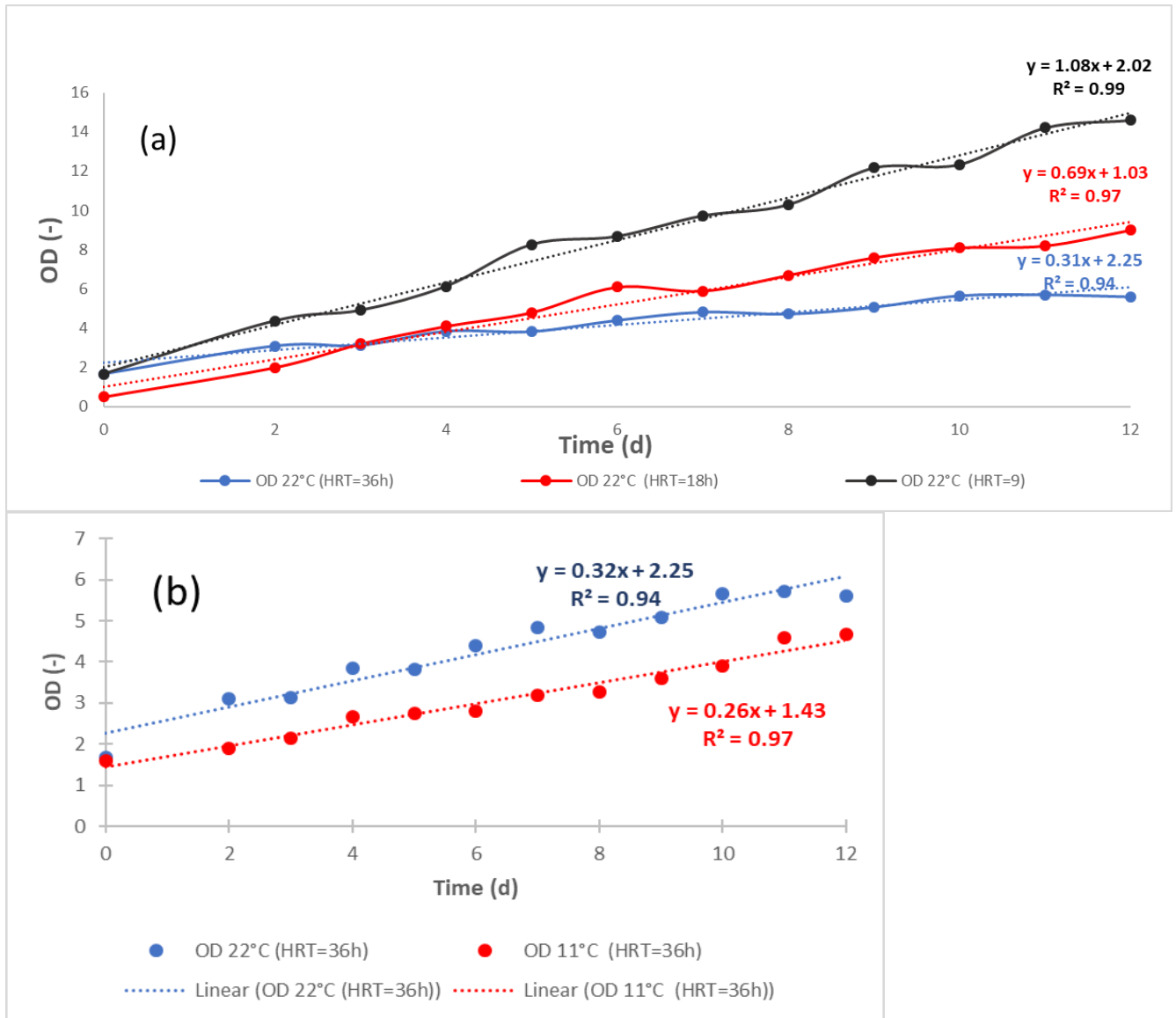
Appendices

Appendix A. Supplementary material for chapter 3

Fed-batch tests setup at 22°C (a) and 11°C (b)
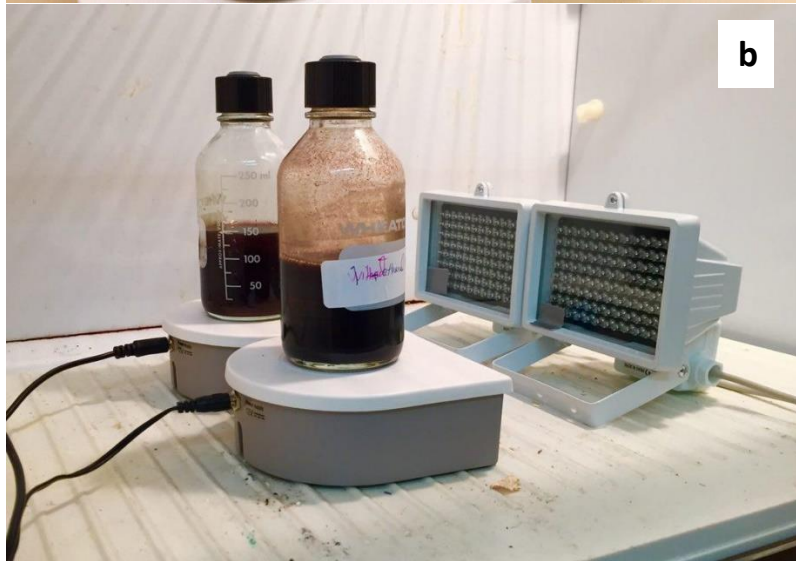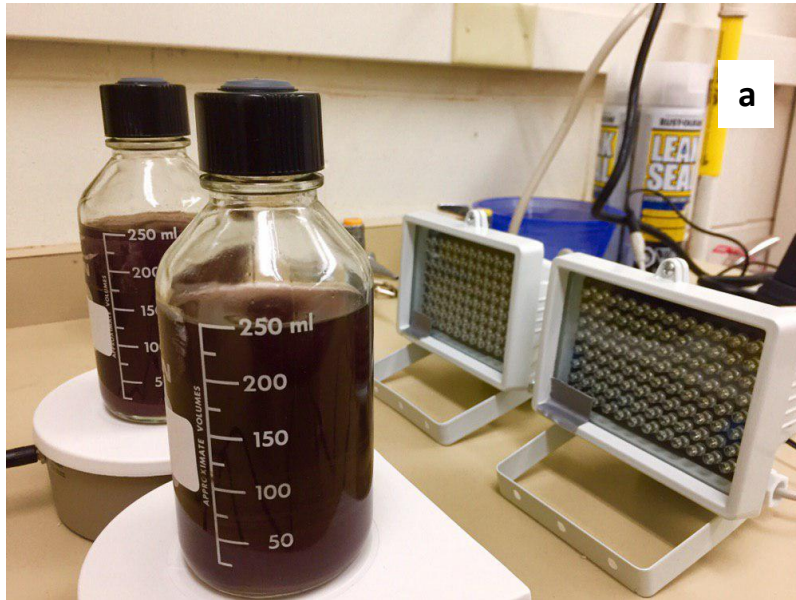
The effect of different HRTs (36 h, 18 h, and 9 h) (a) and low temperature (b) on the PPB growth

Appendix B. Supplementary material for chapter 4

Fed-batch tests setup at low IR light intensity of 3 Wm^{-2}

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