High rate algal pond for greywater treatment in arid and semi-arid areas

Submitted by
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ABSTRACT

In recent years, large scale algal production has received lot of consideration due to the ability of algae to grow extremely rapidly and to accumulate a high quantity of lipid in their cells for biofuel production. For this purpose, algal photosynthetic growth is required and finding an economical and reliable method to produce and harvest algae feedstock remains a challenge. Thereby, apart from closed reactors used for algal production, the use of open ponds also known as high rate algal ponds which integrate wastewater treatment is nowadays emerging. For large scale biofuel production, HRAPs treating wastewater are recognized to be low cost than closed photobioreactors due to the easy operation and cost-effective construction.

While the concern of algal biofuel production and wastewater treatment is currently increasing, this thesis focuses on the ability of HRAP to treat greywater and produce resources for agricultural activities in urban and peri-urban areas of arid and semi-arid countries. To tackle the increasingly severe issues related to water scarcity and domestic wastewater treatment in these areas, a greywater treatment system based on high rate algal pond (HRAP) was developed. As the conventional ponds, this technology is low cost, simple to build and operate. The HRAP is able to provide efficient wastewater treatment because of the assimilation of the wastewater nutrients into the algal biomass. Resources like biomass and energy can be recovered from the wastewater treatment for beneficial use. However, some negative points regarding the implementation of HRAP concern the large land area requirements and the washout of algae from ponds which increases the total suspended solids (TSS) concentration in the effluent. For an efficient wastewater treatment and recovery of resources usable in agriculture, the main purpose of this work consists to find operating strategies of HRAP leading to effective production and harvest of high settleable algal biomass.

At a laboratory scale and under tropical conditions, several reactors simulating HRAP were set and various operated parameters based on the hydraulic retention time (HRT), solid retention time (SRT) and algal recycling were applied. In both reactors, the temperature was kept at 30±2 °C, the mixing of the reactor was performed to avoid algae sedimentation and LED lamps gave photosynthetic photon density varying from 430-550μmol^{-2}s^{-1} at the surface

of the pond. The water qualities of synthetic greywater with their average values ±SD were: pH (6.76±0.45); T-N (12.41±3 mg/L); T-P (5.26±0.4 mg/L); TOC (22.69 mg/L). Samples withdrawn from influent tanks, HRAP, SBRs, CFRs and corresponding effluents tanks were collected once to twice per week and immediately analyzed. Total suspended solids (TSS) together with settleable solids were determined. Nitrogen and phosphorus species such as ammonium- nitrogen, nitrite, nitrate, total nitrogen, soluble reactive phosphorus and total phosphorus (T-P) were measured. As the treated effluent will serve for irrigation purpose, inactivation of E.coli in the system was also investigated.

Chapter 1

A review of the wastewater and greywater management in urban areas of arid and semi-arid areas was made and followed by an analysis of greywater treatment options in urban areas of arid and semi-arid countries. Based on the results of the situation assessment, a treatment option using high rate algal pond technology and allowing resources recovery for agriculture was proposed.

Chapter 2

The configuration of the greywater treatment system used in this section consists of a HRAP which is a photosynthetic reactor followed by an algal settling pond (ASP). In this system, an HRT of 8 days and various SRT of 10, 15 and 20 days were set. The SRT and the recirculation of algae had an effect on the self-granulation and settleability of the algae. In fact, by recirculating the algae, the dominant algal species could be maintained and higher settleability and removal efficiency of the algae could be achieved. Further, operating long SRT of 20 days has increased the TSS concentration in HRAP due to the low TSS concentration caused by the mixture washout during long SRT. For operation under a short SRT of 10 days, efficient algal removal (86%) together with NH_4 +-N and $PO_4{}^{3-}$-P removal (84% and 55% respectively) were achieved. In the ASP, at SRT of 10 days, settleable algae were dominant and the selection has occurred by simple gravity sedimentation.

Chapter 3

To investigate the selection mechanism of self-flocculated algae, the experiments were carried out by using 3 replications of sequencing batch reactors (SBR) and 3 other replications of continuous flow reactors (CFR). The three SBRs which simulated HRAP with algae recirculation were operated at a HRT of 10 days and SRT of 20 days. In the three other CFRs which simulated HRAP without algae recirculation, experiments were carried out using same HRT and SRT of 20 days. Moreover, the effects of the algae recirculation, HRT and SRT control were investigated. Despite operation with the same solid retention time (SRT) and the similarity of the algal growth rate found in both SBRs and CFRs, the algal productivity was higher in the SBRs owing to the short HRT of 10 days in these reactors. Further, in contrast to CFR, the operation of HRAP under batch mode has enhanced the selection of settleable algae through the sedimentation process. It was also found that under similar operating and physical conditions in the SBRs and CFRs, the control of the algal productivity and independent control of HRT and SRT were achieved. The comparison of SBRs and CFRs on their performance to remove the ammonium-nitrogen and T-N has shown that the SBRs presented greater capacity of removal efficiency during all the experimental period. In both reactors, more nitrogen than phosphorus was removed and the concentrations of dissolved phosphorus in the effluents from SBRs were lower. The operation of short HRT and the effect of sedimentation applied in SBRs resulted in a higher algal concentration and thus promoted nitrogen and phosphorus removal by assimilation into algal biomass and sedimentation processes.

Chapter 4

This chapter focuses mainly on the production of resources from the high rate algal pond (HRAP) to serve the agriculture in arid and semi-arid areas. Furthermore, operating strategies for the application of HRAP were indicated. Experiments were achieved by using the systems described in Chapters 2 and 3.

The nitrogen and phosphorus balance were established during the period when the algal growth in the reactors was not subject to change. Contrary to the CFRs where HRT and SRT were similar, in the SBRs, the contribution of the excess algae withdrawn from the reactors has led to the increase of nitrogen exiting the system.

Operating strategies of HRAP to produce settleable algae and treated water for irrigation were suggested. It was proposed that for urban agricultural irrigation, the selection of appropriate hydraulic conditions (long HRT and long SRT) can be implemented to meet different needs. In contrast, the operation of short HRT and short SRT provide effluent with a lower nutrient concentration which can be discharged into reservoirs. The recovered algal biomass from the HRAP could be used either as fertilizer or used as a livestock feed supplement. Focusing on conventional HRAPs, this method might be applied to achieve efficient productivity or removal of the algal biomass.

Chapter 5

The reuse of the greywater for irrigation purpose presents many challenges, including the risk of pathogen infection. In HRAP, algae play an essential role in the process of pathogen removal by raising the pH and dissolved oxygen concentration which favour inactivation of bacteria. The main objective in this chapter was to investigate disinfection processes occurring in HRAP. Therefore under a tropical climate, series of batch experiments were set up to evaluate the potential of algal sedimentation and other pathways of *E. coli* and coliphage (MS2 and Qβ) inactivation.

It was found that the natural decay of bacteria was dominant and UV irradiation was effective. However the recovery of light-damaged cells at night should be considered. MS2 and Qβ removal was not effective when pH was neutral. However when pH was increased to 9, the reduction of 4 log units was observed.

Chapter 6

This chapter discusses the conclusions drawn in this thesis and reports on areas that need further research.

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Chapter 1 : General introduction and literature review

1.1. Background

Arid and semi-arid areas are defined as areas falling within the rainfall zones of 0-300 mm and 300-600 mm, respectively (FAO, 1987). According to the United Nations Environment Management Group (2011), these zones cover approximately 40% of the world's land area and support two billion people, 90% of whom live in developing countries. In Africa, the Sahel region represents the transitional zone between the hyper-arid Sahara desert and the belt of humid savannas (Figure 1.1). In these areas with low availability of fresh water resources (World Bank, 2014), the urban population growth is increasing (Figure 1.2) and the water scarcity becomes an obstacle to the development. For instance, in Ouagadougou (Burkina Faso), large part of the population do not have access to potable drinking water and irrigation is restricted owing to limited water resources (MAHRH, 2003). Moreover, in the urban and peri-urban areas of this country due to the high rate of population growth, water consumption is rising and consequently the volume of wastewater discharged is also increasing. The wastewater refers to the water supply of the community after it has been used in a variety of applications from residences, institutions, commercial and industrial establishment (Tchobanoglous et al., 2003). Domestic wastewater emanating from water closets, bathroom, kitchens and laundry in residences may or may not include storm water or run-off rainwater.

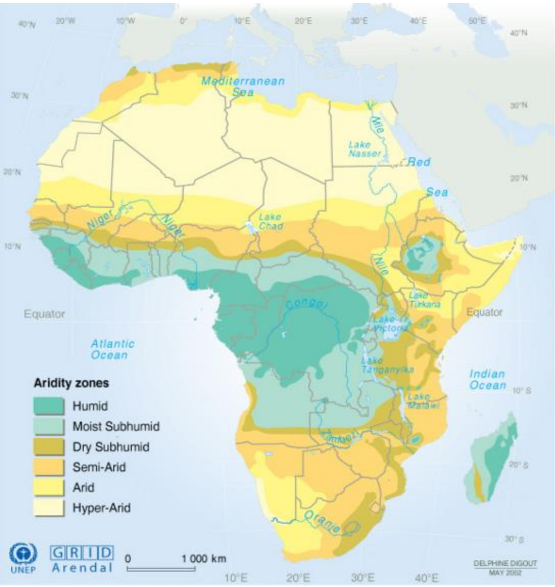


Figure 1.1. Aridity zones in Africa (WMO and UNEP, 2001)

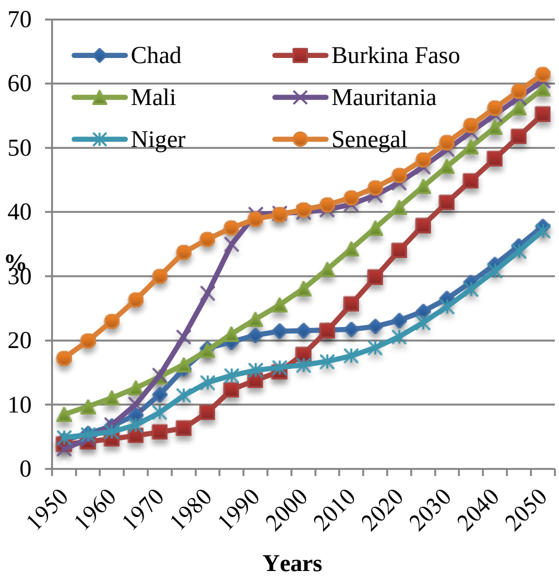


Figure 1.2. Percentage of population residing in urban areas of countries in the Sahel (United Nation, Department of Economics and Social Affairs, Population Division, 2012)

1.1.1. Wastewater and greywater management in urban areas of arid and semi-arid regions: case of Ouagadougou (Burkina Faso)

Burkina Faso is a country of the Sahel region situated in the inland of Western Africa and is among the poorest countries in the world, ranked 183 out of 186 countries in United Nations‘ Human Development Report (UNDP, 2013). The country is experiencing continual problems with desertification and is on the brink of experiencing water scarcity. In addition, most of the household does not have access to sanitation services. In Ouagadougou, the capital city, the existing centralized sewerage was designed to serve only the administrative, university and industrial areas (Vezina, 2002) and most households especially low-income households lack other options for greywater management.

What is greywater?

The wastewater from baths, showers, hand basins, washing machines and dishwashers, laundries, kitchen sinks and ablutions excluding wastewater from the toilet is known as greywater (Dixon et al.,, 1999; Eriksson et al., 2002; Ledin et al., 2001; Ottoson and Stenstrom, 1997; Ahmed, et al., 2008) . As a result of the absence of a sewerage system, households without sanitary facilities and in poor communities discharge greywater in their surroundings. Consequently, these practices present potential risks to public health, local economy and living conditions.

To improve sanitation conditions of the city, a proper greywater management, which includes collection, treatment and reuse or disposal is required. Moreover, by treating greywater, the input of nutrients in nearby water bodies is limited and eutrophication is thereby prevented. Despite the positive impact of the greywater management on public health and living conditions, in recent years, greywater is regarded as a valuable resource and not as a waste.

In developing countries where greywater treatment is recommended and implemented, the reuse of treated greywater for irrigation purposes is gaining importance (Morel, 2005). For agriculture purpose in arid and semi-arid areas the World Health Organization (2006) pointed out several factors leading to that trend:

- The scarcity of alternative water sources for irrigation;
- The high cost of artificial fertilizer;
- The demonstration that risks and soil damage are minimal, if the necessary precautions are taken;
- The high cost of advanced wastewater treatment plants;
- The socio-cultural acceptance of the practice;
- The recognition by water resource planners of the value of the practice.

As stated previously, the wastewater management system in Ouagadougou relies on a centralized wastewater treatment plant, leaving 5% of the population use improved latrines or septic plant (Vezina, 2002). Thereby, a large amount of mixed wastewater and greywater is separated locally and this situation provides an opportunity to achieve on-site greywater treatment which should be easier than the treatment of mixed wastewater (Gajurel et al., 2003). Despite the improvement of public health in urban areas of industrialized countries, centralized approach to wastewater treatment was found to be unsuitable for developing countries, particularly in arid zones. In fact, this approach needs enormous investment, operating and maintenance costs (Morel, 2005). As for the decentralized approach, Morel and Koottatep (2003) indicated that this on-site greywater treatment remained the only appropriate alternative to providing a hygienically safe environment to poor communities. By using small-scale greywater treatment systems, the reuse of treated greywater near the location where water was used initially is ensured and this enables to prevent water shortage. Further, greywater often contains valuable nutrients for gardening and irrigation and as a consequence there is no need to buy expensive mineral fertilizer.

1.1.2. Greywater production and characteristics

In urban areas, the quantity of greywater production depends on the water consumption, habits of the residents and losses due to absorption or evaporation (Ghaitidak and Yadav, 2013). In low-income areas with water scarcity, water consumption can be as low as 20–30 liters per person and day (Ridderstolpe, 2004 and Morel and Diener, 2006). In general, 50-80 % of total water consumption represent the production of greywater (Al-Jayyousi, 2003 and Jamrah, et al., 2011). However, this figure drops significantly in arid, semi-arid areas and locations with lower level of water supply, the greywater production is decreasing. For example, as mentioned Raude et al., (2009), the daily greywater production in households of Nakuru Municipality (Kenya) was 57-72L/d .

The greywater is generally divided into three types (kitchen, bathroom and laundry) and their characteristic is highly influenced by the lifestyle and the choice of household chemicals. The contaminants include suspended solids, pathogens, nutrients, grease and also organic micro-pollutants (Elmitwalli and Otterpohl, 2007).

Greywater contaminants in urban slums and industrialized countries were reviewed by Katuzika et al., (2012). The authors stated that high concentrations of easily degradable organic material substances from cooking, residues from soap and detergents were found in household greywater. Other than that, the proportion of pathogens in greywater is generally low but can be increased due to fecal contamination and the highly biodegradable organic matter content that promotes growth of pathogens. Greywater generally has low and varying concentrations of nutrients. Nitrogen concentrations are low and result from showering and washing (Jefferson et al., 2004). In developing countries, phosphorus concentrations depend mainly on the type of detergents used in kitchens.

1.1.3. Wastewater treatment options in urban areas of arid and semi-arid regions of Africa

Before being reused or disposed greywater is treated depending on the economic aspects, the types of contaminants and the required effluent quality. For the greywater treatment in Ouagadougou, the ONEA recommended the use of leach pits. The construction of leach pits costs around 100,000 FCFA (around 210 USD) and majority of the population cannot afford it.

Review of several greywater treatment systems lead by Ghaitidak and Yadav, 2013 and Morel, 2005 have shown benefits and drawbacks of each treatment technology. From this overview of treatment systems in developing countries the same authors recommended an anaerobic treatment followed by an aerobic system (with post-disinfection, if needed) for the treatment and reuse of greywater. In the 2iE (International institute for water and environmental engineering) campus in Ouagadougou, the pilot plant scale aims to treat greywater and includes successively an anaerobic pond, a HRAP and an algal settling pond.

1.1.4. Reclaimed greywater for irrigation purpose

In this thesis, based on the climatic conditions and the issues found in large scale of farming activities in urban areas, we consider treating greywater through a high rate algal pond (HRAP) which is another alternative to compensate the needs of water and fertilizers in urban farming activities. When greywater is used for unrestricted irrigation purpose, agronomics and water quality consideration are required. Table 1.1, adapted from Pescod, (1992) and IDRC & IWMI, (2010) indicates important characteristics that are used in the evaluation of agricultural water quality.

Table 1.1 Water quality for unrestricted irrigation

A. Potential problem: clogging in drip irrigation systems

Constituents	*Unit*	*Recommended maximum values*	*References*
Suspended solids and algal particles	mg/L	<50	Pescod, (1992) and IDRC & IWMI, (2010)
pH	pH unit	<7	

B. Potential problems: Can cause a range of communicable diseases for farmers, traders and food consumers, such as diarrhea, typhoid, dysentery, and cholera, food-poisoning…

Constituents	*Unit*	*Recommended maximum values*	*References*
Helminthes eggs	number/L	<1/L	World Health Organization, (2006)
E. coli	number/100 mL	<1,000 (relaxed to 10,000 for high growing leaf crops or drip irrigation)	

1.1.5. *Potentials of microalgae*

Microalgae are known as one of the oldest living microorganism on the Earth (Lam et al., 2012 and Song et al., 2008). With their high lipid content , they are considered as a feedstock for bioethanol production (Chisti, 2007) and they have ability to fix CO_2 from the atmosphere (Lam and Lee, 2012).

Apart from that, they have a competitive advantage over other terrestrial crops and show potential in bioremediation applications. Several authors have reported the use of microalgae for the removal of main wastewater pollutants : BOD removal (Grobbelaar et al.,1988; McGriff Jr. and McKinney, 1972; Munoz et al., 2004); Nutrients removal (Laliberte et al., 1994; Oswald, 2003 ; McGriff Jr. and McKinney, 1972 ; Nurdogan and Oswald, 1995) and pathogens removal (Mallick, 2002). Moreover, the different removal mechanisms involved have been described and commented by Muñoz and Guieysse, (2006).

1.1.6. *The high rate algal pond*

As discussed earlier, large scale algal production has received lot of consideration due to the ability of algae to grow extremely rapidly and to accumulate high quantity of lipid in their cells for biofuel production. For this purpose, algal photosynthetic growth is required and finding an economical and reliable method to produce and harvest algae feedstock remains a challenge. Thereby, apart from closed reactors used for algal production, the use of open ponds also known as high rate algal ponds which integrate wastewater treatment is nowadays emerging. For large scale biofuel production, HRAPs treating wastewater are recognized to be low cost than closed photobioreactors due to the easy operation and cost-effective construction.

In recent years, the growth and utilization of algae in many applications become very significant. Wastewater treatment using oxidation ponds or waste stabilization ponds (WSPs) have been achieved without considering algal growth. In contrast, the HRAP "in heart" of the advanced pond system (APS) was developed by Oswald at the University of California (Picot et al., 1992) for variable uses of the different by-products from wastewater treatment. For instance, this technology promotes algal growth as well as nutrient removal and serves the wastewater treatment and needs for agriculture (Shilton and Walmsey, 2010). In addition, the use of HRAP leads to minimal odor emission construction and the operating costs are typically 50% of that of mechanical treatment plants. Numerous HRAP have been implemented around the world for several applications in Brazil (Kawai et al., 1984), Egypt (El-Gohary et al., 1991), New Zealand (Craggs et al., 2003).

HRAP are shallow ponds for wastewater treatment with equipment that makes the liquid circulate in loop and characterized by high flow rates. In these systems, aerobic bacteria break down dissolved organic matter and algae take up nutrients and further BOD. HRAP is the second pond in APS and microalgae grow profusely releasing oxygen from water by photosynthesis (WHO and UNEP, 2006 and Ertas and Ponce, 2014).

1.2. Problem statement

Over conventional oxidation ponds, HRAPs have shown several advantages for the treatment of wastewater as well as the recovery of natural resources for potential reuse. However, effluent from HRAP contains high concentration of algae varying from 100 to 400 mg/L, making the removal of algae essential(Sandbank et al., 1974). Apart from chemical based and physical based methods, algae gravity sedimentation represents one of the low cost methods used for the removal of algal suspension. Thereby, the harvest of algal biomass in a cost-efficient way remains a major challenge to the application of this technology (Su et al., 2011) .

1.3. Objectives of the research

While the concern of algal biofuel production and wastewater treatment is currently increasing, this thesis focuses on the ability of HRAP to treat efficiently greywater and produce resources for agricultural activities in urban and peri-urban areas of arid and semi-arid countries. Therefore, by taking into account the local economic and social conditions the main purpose of this work consists to find operating strategies of HRAP leading to effective production and harvest of high settleable algal biomass.

Specific objectives are as follows:

- To investigate the effects of solid retention time (SRT) and hydraulic retention time (HRT) on bio-flocculated algal production and removal.
- To investigate the selection mechanism of self-flocculated algae
- To assess the algal productivity and resource recovery from the HRAP treating greywater
- To investigate the potential of *E. coli and* coliphage removal from HRAP and determine the different mechanisms of inactivation.

1.4. Structure and outline of the thesis

This thesis consists of six chapters. This first chapter gives a brief background of the wastewater and greywater management in urban areas of arid and semi-arid areas. Thereby, based on the results of the situation assessment, a treatment option using high rate algal pond technology was proposed.

Successively, chapters 2, 3, 4 and 5 introduced a series of scientific papers accepted for publication or submitted for review. In the introduction and materials and methods sections of these chapters there is some repetition. To limit the repetition of the chapters, these sections have been condensed.

Chapter 2 describes the effect of operating parameters (HRT and SRT) on the performance of HRAP to produce flocculated algae and to remove efficiently algal biomass from the system. In Chapter 3, mechanisms of settleable algal selection and approaches to upgrade conventional HRAP were discussed. Chapter 4 discusses factors affecting the improvement of algal biomass production and nutrient recovery for potential uses in agriculture. Further, operating strategies for the application of HRAP in view of the reuse of resources in agriculture were recommended. Chapter 5 reports on the ability of HRAP to remove pathogens and the removal mechanisms were also determined.

Finally, the last chapter consists of conclusions and recommendations for future research.

Chapter 2 : Impact of simultaneous algae recirculation and solid retention time control on algal biomass productivity and nutrient removal in a high-rate algal pond

2.1. Introduction

As mentioned in Chapter 1, in arid and semi-arid areas, particularly in low and middle-income countries of Africa, wastewater management is limited to centralized systems and remains a challenge. As a result, most of the populations with low revenue do not have access to adequate sanitation systems. Moreover, owing to limited water resources, irrigation is restricted.

In this chapter, to contribute to the improvement of wastewater management, particularly greywater management, attention was given to high rate algal pond (HRAP) technology, which is appropriate for domestic wastewater treatment. In addition to the information about this technology provided in Chapter 1, it should be noticed that HRAP reproduces the common phenomenon of algal biomass formation in shallow ponds, where a mixing device induces the circulation of algae and nutrients (Christenson and Sims, 2011; Chisti, 2007). The ability of HRAP technology, suggests that the use of microalgae provides an appropriate domestic wastewater treatment (Chen et al., 2003). According to Chen et al., high nitrogen and phosphorus removal efficiency, a low investment cost and simple management are the main benefits of HRAP technology. Moreover, by using *C. vulgaris* for nitrogen and phosphorus removal from wastewater, Aslan and Kapdan (2006) achieved an average removal efficiency of 72% for nitrogen and 28% for phosphorus. On the other hand, the main disadvantages of this low-cost and simple system concern the growth of algae in ponds which increases the suspended solids (SS) concentration in the effluent (Shelef and Kanarek, 1995 Mara et al., 1992).

To improve the HRAP performance for SS removal and resource recovery, we developed an HRAP to enhance the selection of self-flocculated algae. The HRAP was set up using a semicontinuous reactor operated with the recirculation of the settled algae as described by Park et al., (2011). In the research led by these authors, the HRAP was operated with algal recirculation to improve HRAP algal productivity for algal biofuel production. In this study, we have focused on the benefit of HRAP to produce treated effluent for irrigation purpose or disposal in water reservoirs. Thereby, in addition of the recirculation of the settled algae in the HRAP reactor, different solid retention time (SRT) operating conditions were set.

2.2. Materials and methods

All experiments were carried out at the laboratory scale. The system consisted of HRAP followed by an algae settling pond (ASP) as illustrated in Figure 2.1.

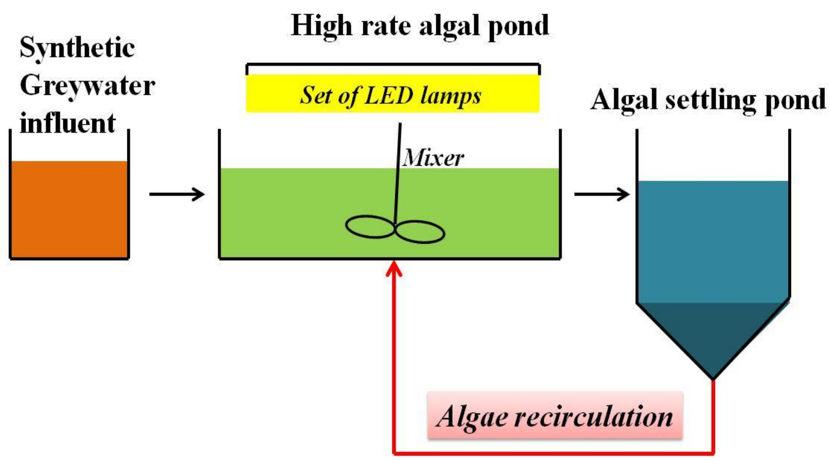


Figure 2.1. Reactor setup of the high rate algal pond

2.2.1. Reactor setup and operation

The surface area, depth and volume of the HRAP were respectively 0.25m^2, 0.105 m and 26 L, and the ASP had a volume of 15 L.

In this system, the effects of algae recirculation and SRT were investigated. For a hydraulic retention time (HRT) of 8 days, synthetic greywater was fed into the photosynthetic reactor by the semi-continuous operation of a feeding pump for 6 hours per day. SRTs of 10, 20 and 15 days in the reactor were achieved by withdrawing a designated amount of the mixed liquor calculated from Equation 2.1.

Equation 2.1. SRT in reactors

$$SRT = \frac{(V_R \times SS_R)}{(x \times SS_R) + (Effluent_{flowrate} \times SS_E)}$$

V_R : Reactor volume (L)

SRT : Solid retention time (d)

SS_R : SS concentration in the HRAP (mg/L)

$Effluent_{flowrate}$: Effluent flow rate (L/d)

SS_E : SS concentration in the effluent

x : Withdrawal volume (L/d)

The ASP, which has a retention time of 5 days, received supernatant from the HRAP, allowed algae sedimentation and produced the final treated effluent.

As schematically defined in Figure 2.1, the recirculation of the settled algae from the bottom of the ASP to the HRAP manually carried out daily. The recirculation ratio, i.e., the ratio of return algae volume to the effluent volume, was 0.5.

The microorganisms originated from the sediment collected from a reservoir in Ouagadougou (Burkina Faso). They were inoculated into a medium (M-11) developed in Japan to culture *Mycrocystis*. The algae were harvested from the medium, and inoculated into the photosynthetic reactor fed by synthetic greywater. Table 2.1 shows the components of M-11 medium.

Table 2.1. Components of M-11 medium (mg/L)

Components	
$NaNO_3$	100
K_2HPO_4	10
$MgSO_4 \cdot 7H_2O$	75
$CaCl \cdot 2H_2O$	40
Na_2CO_3	20
Fe-citrate	6
$Na_2EDTA.2H_2O$	1
pH	8

The attached biomass in the HRAP reactor were controlled by cleaning the reactor's walls before collecting a sample and before recovering in the ASP of the settled algae that was subsequently used for recirculation. Furthermore, protozoan grazers were removed by carrying the mixed liquor to a supersonic disruptor to break down the protozoa bodies. This operation was carried out approximately every two months because of the proliferation of grazers.

The irradiance light in the HRAP was supplied by a set of conventional white light LED lamps (TOSHIBA LDA 6N). The light period was a 12 h light–12 h dark cycle and the set of LED lamps provided a photosynthetic photon density of 430 µmol m^{-2} s^{-1} at the surface of the pond. The wavelength of the photoluminescence spectrum emitted by these LEDs ranged between 400 and 800 nm and exhibited a peak at 465 nm.

The temperature was continuously maintained at 30 ± 2 ºC (average temperature in Burkina Faso), and the reactor water was mixed by mixers (AS ONE and EYELA MDC-NC) to avoid algae sedimentation and to enhance light penetration (Paterson and Curtis, 2010).

2.2.2. Synthetic greywater composition

The water quality index of the synthetic greywater was similar to that used in Raude et al. (2009) for the greywater in peri-urban areas of Nakuru (Kenya). Using a dilution ratio of 400, the synthetic solution was prepared by dissolving the compounds listed in Table 2.2 in 1 L of pure water. The water qualities of the synthetic greywater given as their average values ±SD were: pH (6.76 ± 0.45); [T-N] (12.41±3 mg/L); [T-P] (5.26±0.4 mg/L) and [TOC] (23 mg/L).

Table 2.2. Synthetic greywater composition

Components	**Concentration (g/L)**
Dextrin hydrate	3.68
Bacteriological peptone	8
Extract Ehlrich	2.69
Yeast extract powder	8
KCl	1.6
NaCl	0.8
$MgSO_4 \cdot 7H_2O$	1
KH_2PO_4	8.72
NH_4Cl	11.18
KNO_3	5.686
Fe-citric acid	2.4

2.2.3. Analytical methods

Samples withdrawn from the influent tank, HRAP and effluent were collected once or twice per week and immediately analyzed. The temperature and pH were measured in situ using a pH meter (Horiba, D-52). The photosynthetic photon flux (PPF) was measured at the center of the reactor and at the top of the liquid surface by a photosynthetically active radiation meter (Apogee SE-MQ200). Total suspended solids and settleable solids were determined in accordance with

APHA (2005) using glass fiber filters (Advantec GS-25, 47 mm). Before nutrient analysis, all samples were filtered with membrane filters (Advantec GF-45, 0.45μm). NH_4^+-N, total nitrogen (T-N) and total phosphorus (T-P) were determined by Hach methods 8038, 10071, and 8190 respectively. Nitrite, nitrate and soluble reactive phosphorus were also measured using Hach methods 10019, 10020, and 8114, respectively. The removal efficiency and total productivity of the reactor were calculated using equations 2.2 and 2.3 respectively.

Equation 2.2. Removal efficiency

$$R\,(\%) = \frac{C_{inf} - C_{eff}}{C_{inf}} \times 100$$

C_{inf}: Influent concentration (mg/L)

C_{eff} : Effluent concentration (mg/L)

Equation 2.3. Total productivity

$$P(mg/d) = SS_{mixturewashout} + SS_{supernatant} + \Delta SS_{HRAP}$$

P : Total productivity (mg/d)

$SS_{mixturewashout}$: SS withdrawn from the HRAP (mg/d)

$SS_{supernatant}$: Mass of SS in supernatant (mg/d)

ΔSS_{HRAP} : Variation of SS in HRAP (mg/d)

2.3. Results and discussion

2.3.1. Algae removal and biomass productivity

The algal concentration in the reactor was estimated as the total amount of suspended solids (TSS).

- Algae removal

In the HRAP, an HRT of 8 days and various SRTs were set. The values presented in Table 2.3 show the average of the data points collected during the 85 days of experiments. Additionally, SRTs of 10, 15 and 20 days corresponded respectively to sets of 16, 43, and 26 data points. For each SRT, a period of acclimatization was allowed before the collection of the data.

Table 2.3. Average TSS concentrations and removal efficiencies in the HRAP for different SRTs

	HRAP (mg/L)	**Effluent (mg/L)**	**% TSS removal**
SRT 10 days	103	13	87
SRT 15 days	159	40	75
SRT 20 days	162	37	77

In the HRAP, as presented in Figure 2.2 and Table 2.3, with increasing SRT, the algal biomass concentration gradually increases. The TSS in the HRAP increased with SRT because the TSS concentration of the mixture washout decreased when the SRT increased. Furthermore, the long period of storage of the algae in the reactor could explain its accumulation.

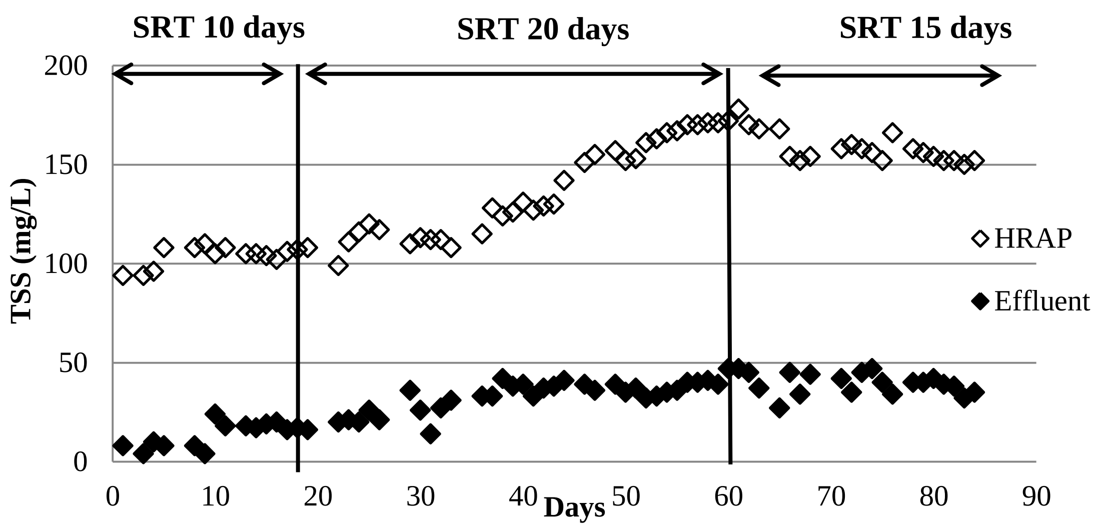


Figure 2.2. Change in TSS concentration in HRAP and effluent with SRT

In the effluent samples, in the case of SRTs of 10, 15 and 20 days, the TSS concentration was always <50 mg/L (Figure 2.2).

The data presented in Figure 2.2 and Table 2.3 attested the enhanced TSS removal efficiency when a shorter SRT of 10 days was set (87 % for SRT of 10 days). In contrast, the algae concentration in the effluent increased when a long SRT was set (TSS removal efficiency of 77% for SRT of 20 days). These results confirmed that for a short SRT of 10 days, settleable algae was dominant inside the ASP, which could be selected and separated from the mixture by simple gravity sedimentation. This resulted in the efficient removal of the algae (Figure 2.3).

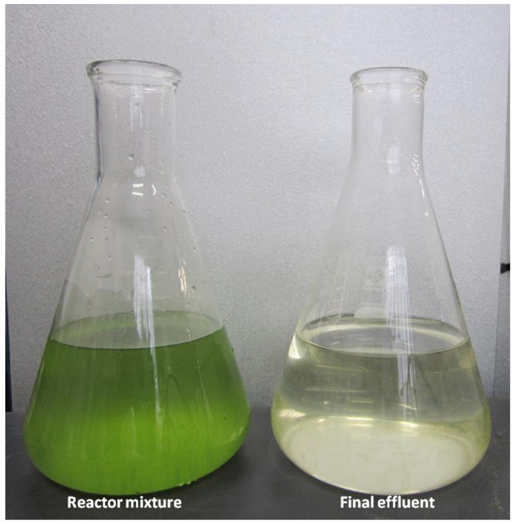


Figure 2.3. View of settleable microalgae culture before and after sedimentation

The left conical flask shows the HRAP mixture and the right conical flask shows the final effluent.

- Algal biomass productivity

Figure 2.4 presents the algal biomass productivity of the HRAP system which was estimated using equation 3. An SRT of 10 days resulted in higher solid production than SRTs of 15 and 20 days. Indeed, when the SRT was 10 days, the amount of the removed excess solid was about four and three times greater than those when the SRTs were 20, and 15 days, respectively.

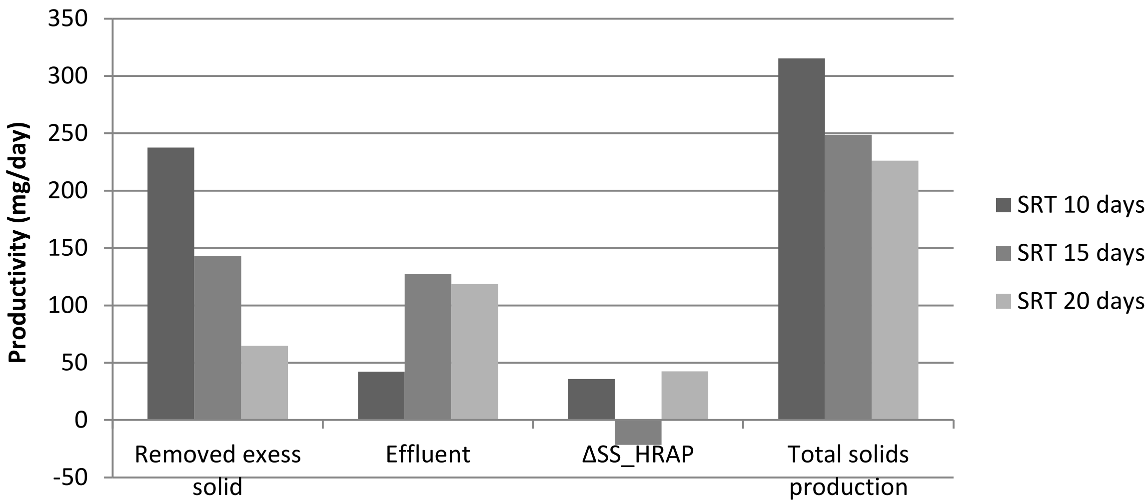


Figure 2.4. Average productivity of removed excess solid, and effluent and total productivity of the HRAP system for different SRTs

Additionally, the process of algae recirculation led to the improvement of many aspect of the reactor. In recent HRAP studies conducted by Park et al. (2011) and Valigore et al. (2012), the performance of the HRAP in reducing the washout occurring at a shorter HRT was discussed. Furthermore, by recirculating the algae, the dominant algal species could be maintained and higher settleability and removal efficiency of the algae could be achieved.

2.3.2. Nitrogen and phosphorus removal

- Nitrogen removal

The results of the experiments on the effect of the SRTs on nitrogen removal are presented in Figure 2.5 and Table 2.4. For a long SRT, the ammonium-nitrogen removal was less efficient than for a short SRT (Figure 5). The ammonium-nitrogen removal efficiencies for SRTs of 10, 15, and 20 days were respectively 86%, 77%, and 44%. Therefore, a lower SRT produced an effluent with a relatively low ammonium concentration. As ammonium-nitrogen was the main nitrogen species found in the feeding (Figure 5), a short SRT also resulted in a higher T-N removal efficiency (88%, 58%, and 54% for SRTs of 10, 15, and 20 days, respectively).

The same tendency was also observed for the removal efficiency of the algae (87%, 75% and 77% for SRTs of 10, 15, and 20 days, respectively), suggesting that the nitrogen can be removed efficiently with an efficient algal removal.

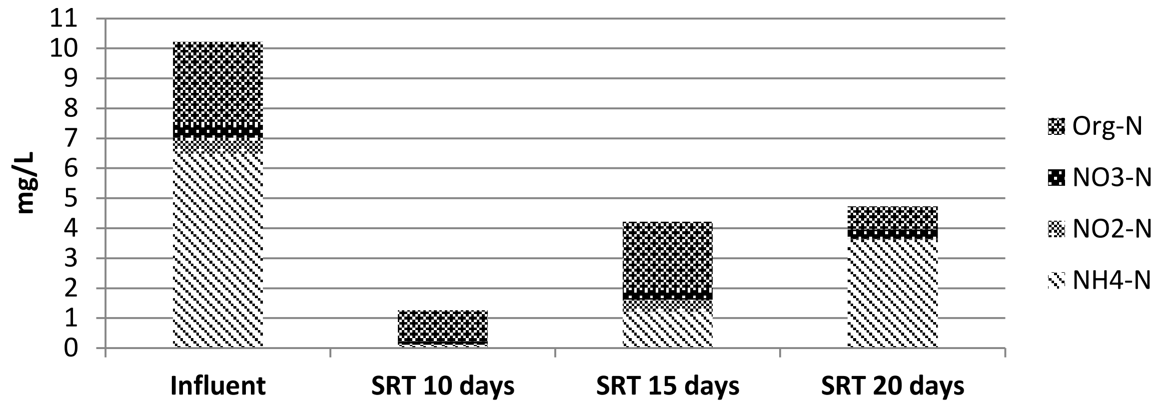


Figure 2.5. Nitrogen concentration and removal from the HRAP for different SRTs

Table 2.4. Effect of SRT on T-N and ammonium removal in the HRAP

SRT (days)	T-N (mg/ L)		NH_4^+-N (mg/ L)	
	Influent	*Effluent*	*Influent*	*Effluent*
10	10.33	1.25	6.37	0.11
15	10.10	4.22	6.61	1.21
20	10.31	4.72	6.60	3.60

In fact, the assimilation of nitrogen by the algal biomass and the sedimentation processes occurred in the HRAP and ASP. These two processes were proposed by Lai and Lam (1997) to be the main mechanisms responsible for the nitrogen removal in HRAPs and waste stabilization ponds (WSPs). Similarly to the nitrogen uptake, sedimentation, volatilization, and nitrification/denitrification are also considered as essential mechanisms of nitrogen removal in WSPs (Craggs, 2010a) and HRAPs (Craggs, 2010a and Garcia et al., 2000). The measurements of nitrogen from the effluent showed that much of the nitrogen removed from the reactor was not removed in the form of oxidized N, owing to the low nitrate concentration (Figure 2.5). This indicates that nitrification and denitrification were not the main processes responsible for nitrogen removal. Since the mean pH in the reactor was less than 8.5, ammonia volatilization through the pond surface might have been limited. Moreover, similar observations were reported by Shilton (1996) and by Su et al. (2012).

- Phosphorus removal

The amount of phosphorus removed from the HRAP was lower than the amount of nitrogen removed. In this regard, El Hamouri (2009) and Shilton et al. (2012) indicated that the amount of phosphorus removed in facultative ponds and HRAPs is commonly quite low.

Since the greywater did not contain large quantities of cations (Fe^{2+}, Al^{3+}, Ca^{2+}, Mg^{2+},…) inorganic phosphate might not have been removed by precipitation in the ASP (Diaz et al., 1994; Nurdogan and Oswald, 1995). Regarding this, Gomez et al. (2000) found significant adsorption of phosphorus on pond sludge containing high concentrations of Fe and Al.

Compared with nitrogen removal, a similar pattern of phosphorus removal was observed for different SRTs. As illustrated in Figure 2.6, PO_4^{3-}-P was more efficiently removed from the system when a short SRT was set. During the short SRT, the efficiency of phosphorus assimilation/sedimentation depended on the algal biomass concentration, which was lower than that when a long SRT was set (Table 2.3).

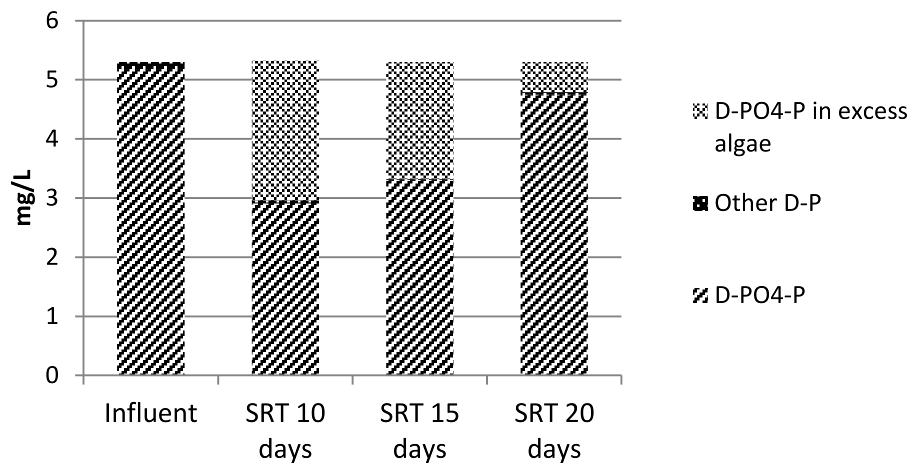


Figure 2.6. Phosphorus concentration and removal for different SRTs

As shown in Figure 2.7, by comparing N and P elimination, much less phosphorus than nitrogen was removed from the reactor during the entire experimental period. This was explained by Redfield (1934) and Craggs (2010b), who observed that algae had a N:P ratio of approximately 15:1. Consequently, the wastewater with a N:P ratio of 4:1 did not contain sufficient nitrogen to enable the complete removal of phosphorus by the assimilation process (Craggs, 2010b; Nurdogan and Oswald, 1995).

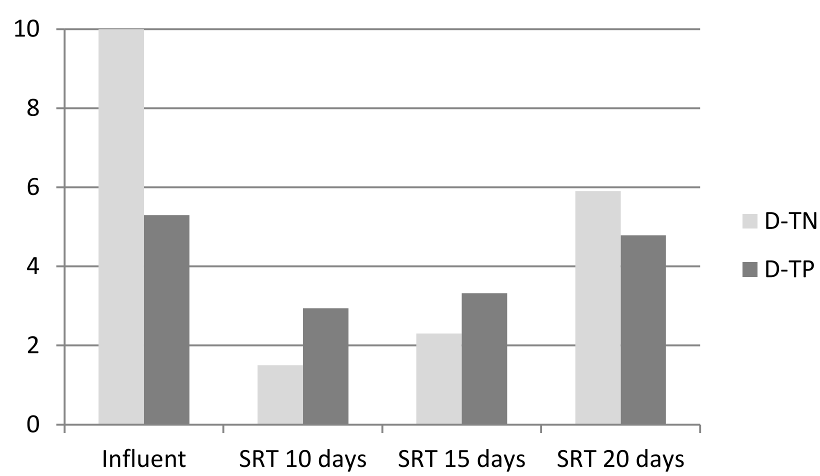


Figure 2.7. D-N and D-P concentrations for different SRTs

2.4. Conclusions

The laboratory-scale experiments confirmed that:

- The solid retention time (SRT) and the recirculation of algae had an effect on the self-granulation and settleability of the algae.
- A short SRT of 10 days enhanced the algal removal efficiency (86%) in the HRAP, while for long SRT of 20 and 15 days, the algal removal efficiency was about 75%.
- A long SRT of 20 days allowed higher algal biomass production than that when a short SRT was set.
- The SRT and the recirculation of algae had an effect on the nutrient concentration
- Higher NH_4^+-N and PO_4^{3-}-P concentrations produced when long SRT of 20 days was operated
- Nitrogen and phosphorus were mainly removed through assimilation/sedimentation processes.
- The HRAP effluent with a high nutrient concentration might be used for irrigation purposes, whereas the effluent with a lower nutrient concentration could be discharged into reservoirs to prevent eutrophication.
- By controlling the SRT, the HRAP was able to produce effluent with both high and low nutrient concentrations

Chapter 3 Control of algal production in high rate algal pond: investigation through batch and continuous experiments

3.1. Introduction

In developing countries covered by arid and semi-arid climate, water, sanitation and hygiene problems are considered as the cause of most diarrheal deaths in the world (88%). Simultaneously , urban agriculture is also growing to feed the growing urban population (UNDP, 2013). As discussed in Chapters 1 and 2, for variable uses of the different by-products of wastewater treatment, a high rate algal pond (HRAP) was operated during this study.

Despite the existing methods to produce high-quality effluent and to improve the production and harvest of bio flocculated algae from HRAP, the present study focuses on the harvesting of algae through the gravity sedimentation process. In this chapter a series of sequencing batch reactors (SBRs) and continuous flow reactors (CFRs) were operated. The SBRs represented HRAP operated intermittently with algal sedimentation whereas; the CFRs indicated the conventional HRAP operated continuously. We discussed the selection mechanisms and the efficiency of algae separation by varying the hydraulic retention time (HRT) and solid retention time (SRT) in replications of batch and continuous experiments. Thereby, algal productivity and nutrient removal efficiency for each case was assessed and compared.

3.2. Materials and methods

All experiments were carried out on a laboratory scale and the collected data will be used for the operation of the pilot scale implemented in the 2iE campus in Ouagadougou (Burkina Faso). The systems operated in the laboratory scale consisted of a series of sequencing batch reactors (SBRs) and continuous flow reactors (CFRs).

3.2.1. Reactor setup and operation

Figure 3.1 illustrates the operation of SBRs and CFRs. To ensure the reliability and validity of the results, the SBR and CFR configurations consisted of an experimental system including three replications of SBR and three others for the CFRs. The CFRs and SBRs were constructed from PVC, had a cylindrical shape, a capacity of 11.5 L and a depth of 0.4 m.

In both reactors, the temperature was continuously maintained at 30 ± 2 ºC (the expected temperature in tropical countries), and the water was mixed using mixers (AS ONE and EYELA MDC-NC) to avoid algal sedimentation and to enhance light penetration (Paterson and Curtis 2010). The irradiance light in the reactors was supplied by conventional white LED lamps (TOSHIBA LDR9L-W). A 12h light–12h dark cycle was employed and the LED lamps provided a photosynthetic photon density of 430-550 μmol m^{-2} s^{-1} at the surface of the pond. The wavelength of the photoluminescence spectrum emitted by these LEDs ranged between 400 and 800 nm and exhibited a peak at 465 nm.

The three SBRs were operated with a hydraulic retention time (HRT) of 10 days and a solid retention time (SRT) of 20 days. The SRT was controlled by withdrawing a designated amount of the mixture in the reactor calculated from equation 1. During each day, the reaction (feeding, mixing), settling and discharging (idle) times were 17.5, 6 and 0.5 h, respectively.

Equation 3.1. SRT in reactors

$$SRT = \frac{(V_{\mathrm{R}} \times TSS_{\mathrm{SBR}})}{(x \times TSS_{\mathrm{SBR}}) + (Effluent_{\mathrm{flowrate}} \times TSS_{\mathrm{E\_SBR}})}$$

V_{R} : Reactor volume (L)

SRT : Solid retention time (d)

TSS_{SBR} : TSS concentration in SBR (mg/L)

$Effluent_{\mathrm{flowrate}}$: Effluent flow rate (L/d)

$TSS_{\mathrm{E\_SBR}}$: TSS concentration in effluent from SBR (mg/L)

x : Withdrawal volume (L/d)

The three CFRs were continuously fed with synthetic greywater. Experiments were carried out using the same HRT and SRT of 20 days, and supernatants (E_CFRs) were withdrawn continuously (Figure 1.b). The configuration of the CFRs, such as the location of the outlet, was the same as that of the SBRs to equalize the hydraulic conditions.

For both reactors, the HRT used for the operation corresponded to that in previous studies (Oswald, 1986; Garcia et al., 2000) and the expected value in practical operation.

The attached biomass in all the reactors was removed by cleaning the reactor's walls before collecting each sample. Furthermore, protozoa grazers were removed by transferring the mixed liquor to a supersonic disruptor to break down the protozoa bodies. This operation was carried out approximately every 2 months because of the proliferation of grazers.

The algal culture was originated from pond water sediment collected from the wastewater treatment plant in 2iE campus (International Institute for Water and Environmental Engineering) in Ouagadougou (Burkina Faso). 0.5 L of the sediment was first grown at room temperature and under illumination in an Erlenmeyer flask containing 0.5 L of synthetic greywater. Cultures were renewed weekly and both reactors were inoculated with 0.5L of the grown cultures after two weeks.

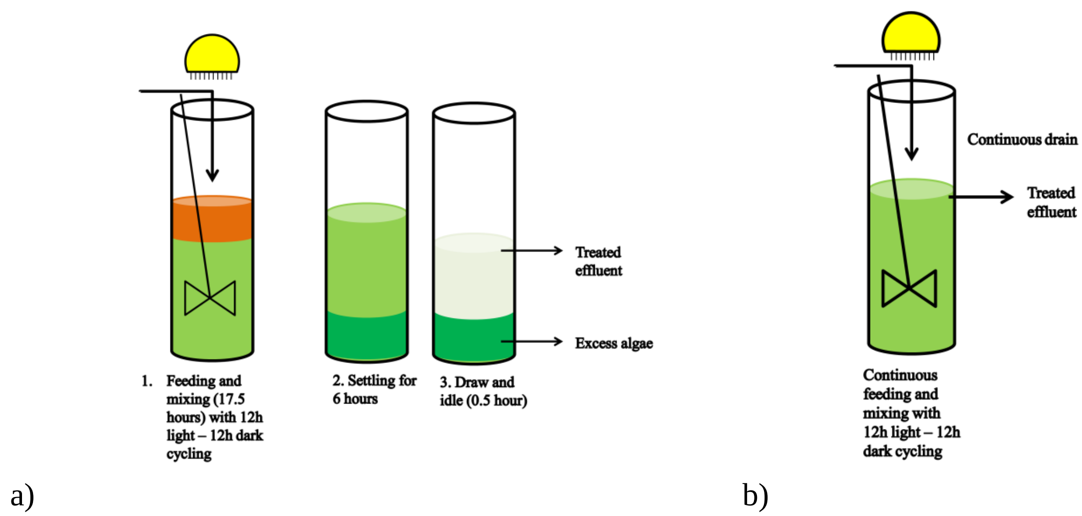


Figure 3.1. Daily operations of reactors (a) Sequencing batch reactor (SBR). (b) Continuous flow reactor (CFR).

3.2.2. *Synthetic greywater composition*

Synthetic greywater was used throughout the experiments. Both reactors were fed with a similar feed of synthetic greywater (Figure 3.1) with a composition similar to that used in Raude et al. (2009) and that was obtained from greywater in peri-urban areas of Nakuru (Kenya). Using a dilution ratio of 400, the synthetic solution was prepared by dissolving the following compounds in one liter of pure water: dextrin hydrate (3.68 g), bacteriological peptone (8 g), fish meal (2.69 g), yeast extract powder (8 g), KCl (1.6 g), NaCl (0.8 g), $MgSO_4 \cdot 7H_2O$ (1 g), KH_2PO_4 (8.72 g), NH_4Cl (11.18g), KNO_3 (5.68 g) and Fe-citric acid (2.4 g). The chemical characteristics of the synthetic greywater with their average values ±SD are given in Table 3.1.

Table 3.1. Main characteristics of the synthetic greywater (mean ± SD)

Characteristic	**Mean ± SD**
pH	6.76 ± 0.45
T-N (mg/L)	12.41 ± 3.00
T-P (mg/L)	5.26 ± 0.40
TOC	21.83

3.2.3. Analytical methods

Samples withdrawn from the influent tanks, SBRs, CFRs and corresponding effluent tanks were immediately analyzed. The temperature and pH were always measured at the moment of collection using a pH meter (Horiba, D-52). The photosynthetic photon flux (PPF) was measured at the center of the reactor and at the top of the liquid surface with a photosynthetically active radiation meter (Apogee SE-MQ200). The total amount of suspended solids was determined in accordance with APHA (2005) using glass fiber filters (Advantec GS-25, 47 mm). Settleable algae were measured by the gravimetric method (APHA, 2005) and the percentage of settable algae was calculated according to the equation 2. Before nutrient analysis, samples were filtered with membrane filters (Advantec GF-45, 0.45µm). NH_4^+-N, total nitrogen (T-N) and total phosphorus (T-P) were determined by Hach methods 8038, 10071 and 8190, respectively. Nitrite, nitrate and soluble reactive phosphorus concentrations were also measured using Hach methods

10019, 10020 and 8114, respectively. The removal efficiency and total productivity of each reactor were calculated using Equations 3.2-3.6.

Equation 3.2. Percentage of settleable solids

$$Settleable\ solids\,(\%) = \left(\frac{TSS_{\mathrm{Reactor}} - non\,settleable\ solids}{TSS_{\mathrm{Reactor}}} \right) \times 100$$

TSS_{Reactor}: TSS concentration of the reactor's mixtures (mg/L)

$non\,settleable\ solids$: TSS concentration of the supernatant after 1 hour of sedimentation (mg/L)

Equation 3.3. TSS removal efficiency

$$R_{\mathrm{TSS}}\,(\%) = \frac{TSS_{\mathrm{Reactor}} - TSS_{\mathrm{effluent}}}{TSS_{\mathrm{Reactor}}} \times 100$$

R_{TSS} : TSS removal efficiency (%)

TSS_{effluent}: Effluent TSS concentration (mg/L)

Equation 3.4. Nutrient removal efficiency

$$R_{\mathrm{nutrient}}\,(\%) = \frac{C_{\mathrm{inf}} - C_{\mathrm{eff}}}{C_{\mathrm{inf}}} \times 100$$

R_{nutrient}: Nutrient removal efficiency (%)

C_{inf} : Influent concentration (mg/L)

C_{eff} : Effluent concentration (mg/L)

Equation 3.5. Total algal productivity of SBRs

$$P_{\mathrm{SBR}}(mg/d) = X_{\mathrm{Excess\ algae}} + X_{\mathrm{E\_SBR}} + \Delta X_{\mathrm{SBR}}$$

P_{SBR} : Total productivity of SBR (mg/d)

$X_{\mathrm{Excess\ algae}}$: Algal biomass withdrawn from the SBR (mg/d)

$X_{\mathrm{E\_SBR}}$: Algal biomass in effluent from SBR (mg/d)

ΔX_{SBR} : Variation of algal biomass in SBR (mg/d)

Equation 3.6. Total algal productivity of CFRs

$$P_{\mathrm{CFR}}(mg/d) = X_{\mathrm{E\_CFR}} + \Delta X_{\mathrm{CFR}}$$

P_{CFR} : Total productivity of CFR (mg/d)

$X_{\mathrm{E\_CFR}}$: Algal biomass in effluent from CFR (mg/d)

ΔX_{CFR} : Variation of algal biomass in CFR (mg/d)

The algal growth rate in the SBRs and CFRs was evaluated as follows.

Equation 3.7. Algal growth rate in SBRs

$$\mu_{\mathrm{SBR}} = \frac{\Delta X_{\mathrm{SBR}} + X_{\mathrm{E\_SBR}} + X_{\mathrm{excessalgae}}}{X_{\mathrm{SBR}}}$$

μ_{SBR} : Algal growth rate in SBRs and CFRs (d^{-1})

Equation 3.8. Algal growth rate in CFRs

$$\mu_{\mathrm{CFR}} = \frac{\Delta X_{\mathrm{CFR}} + X_{\mathrm{E\_CFR}}}{X_{\mathrm{CFR}}}$$

μ_{CFR}: Algal growth rate in CFRs (d^{-1})

3.3. Results

3.3.1. Algal biomass productivity in SBRs and CFRs

The total algal productivity in the SBRs represents the biomass productivities of the excess algae and the effluent. For the CFRs, it represents the biomass productivity of the effluents. The SRT was the same i.e., 20 days for both reactors, and in the SBRs, the excess algae corresponded to the amount of the mixture withdrawn and was evaluated by using equation 1. In the CFRs, the HRT of 20 days enabled the self-control of the SRT 20 days, and the mixture was continuously withdrawn.

As given in Table 3.2, during the steady-state region in Figure 3.2 (Day 33 to Day 44), the mean total areal productivity of the algae in the SBRs was three times higher than that in the CFRs (3.6 g/m^2/day in the SBRs and 1.2 g/m^2/day in the CFRs). Then, the TSS concentrations and the growth rate of the mixture in each reactor were estimated to clarify the reason for the difference in the observed algal productivity.

Table 3.2. Average algal production in SBRs and CFRs

Averages	**Effluent (mg/d)**	**Excess algae (mg/d)**	**Total productivity (mg/d)**	**Total areal productivity ($g/m^2/d$)**
SBRs	3.4±3.6	104.5±17.6	108	3.60
CFRs	35.7±12.3	-	36	1.20

- Algal concentrations and algal settleability

The algal productivity was affected by the algal concentration which was determined in terms of TSS concentration (Table 3.2). The mean algal concentration was determined during the steady-state period, and the results in Figure 3.2 confirmed that the algal concentration was higher in the SBRs (190 mg/L) than in the CFRs (130 mg/L). After the steady state period in the SBRs, a decrease in TSS concentration was observed. This might be due to the effect of the photo inhibition occurring at a small depth, leading to a decrease in the number of large algae flocks.

Experiments determining the percentage of settleable solids revealed that the percentage of settleable algae was significantly higher in SBRs (97±2 %) than one in CFRs (83±14 %).

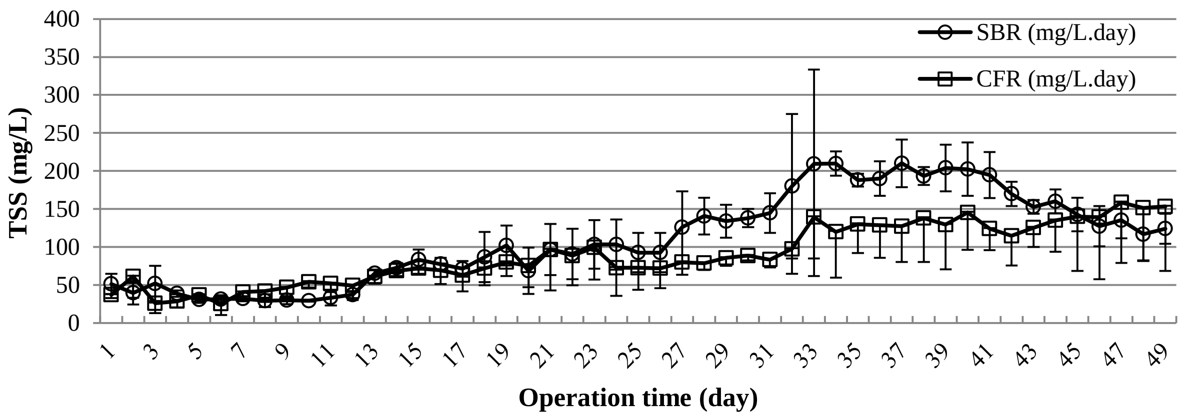


Figure 3.2. Average algal concentration in the mixture in each reactor

- Algal growth rates

The mean algal growth rates in the SBRs and CFRs were obtained using Equations 3.7 and 3.8, respectively. The results are shown in Figure 3.3, which reveals that the algal culture had similar growth rates under batch and continuous operations. The operation of the SBRs and CFRs with the same SRT may explain this similarity of the algal growth rate.

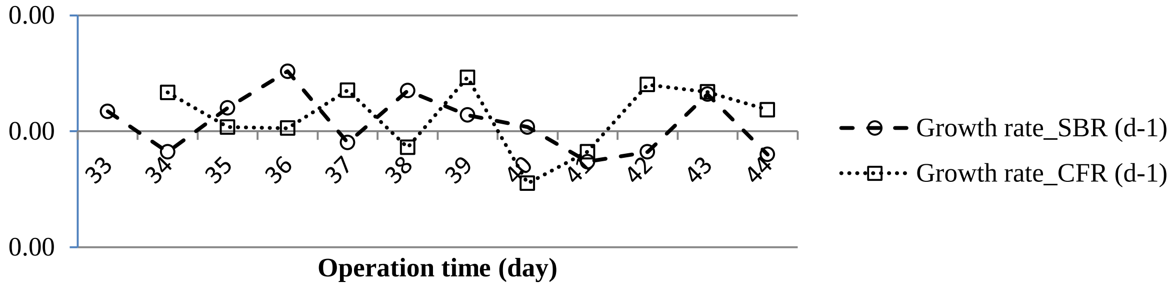


Figure 3.3. Mean algal growth rates in SBRs and CFRs.

3.3.2. Nutrient concentrations

The concentrations of nitrogen and phosphorus before and after the treatment are given in Table 3.3.

Table 3.3. Nutrient concentrations (mean±sd) in influent and effluent for each reactor

	Influent	**E-SBR**	**E-CFR**	**%removal_SBR**	**% removal_CFR**
T-N	14.12±2.23	5.41±0.93	8.59±1.36	58.77±7.02	37.74±9.49
NH4-N	7.16±0.97	3.29±0.39	5.57±0.38	53.39±5.15	20.07±5.40
T-P	5.03±0.63	3.51±0.15	4.52±0.20	30.19±3.23	8.02±3.97
PO4-P	4.24±0.45	2.69±2.69	4.16±0.17	33.36±6.82	1.14±4.17

Abbreviations: E-SBR: effluent from SBR; E-CFR: effluent from CFR

Larger amounts of NH_4^+-N and T-N were removed from the SBRs, than from the CFRs during the entire experimental period. The nitrogen from the reactors was not eliminated in the form of oxidized N. In fact, the nitrate concentrations throughout the steady-state period of the cells were insignificant (average values of 0.33±0.08 mg/L and 1.08±0.43 mg/L in effluents from SBRs and CFRs respectively). The pH was measured in the SBRs and CFRs and the corresponding average did not exceed 8.5. In all reactors, more nitrogen than phosphorus was removed and the concentrations of dissolved phosphorus in the effluents from the SBRs were lower than those in the effluents from the CFRs.

TOC removal in HRAP has been investigated in previous studies of our research group. The results have shown that the TOC removal efficiency was around 61 %.

3.4. Discussion

3.4.1. Algal biomass productivity in SBRs and CFRs

The SBR simulates an HRAP with algal recirculation and the CFR simulates a conventional HRAP without algal recirculation in our experiment.

In SBRs and CFRs where the percentages of settleable algae were higher than 80%, the bio flocculation of algal biomass occurred naturally. On the other hand, by introducing algal sedimentation in SBRs, the selection of settleable algae was efficient, thus increasing the percentage of settleable algae in SBRs. In CFRs, where continuous operations were conducted, low percentage of settleable algae was observed probably due to the absence of algal sedimentation.

As shown in the results section, the growth rates of the SBRs and CFRs were similar. However, the areal algal productivity and algal concentration were higher in the SBRs than CFRs in spite of the same SRT, as shown in Table 3.4.

Table 3.4. Average algal productivity, TSS concentration and growth rate of SBRs and CFRs

	Areal productivity ($g/m^2/d$)	**Algal concentration in reactors (mg/L)**	**Algal growth rate (d^{-1})**	**Algal productivity (L^{-1} of influent)**
SBR	3.60	190	0.02	93.9
CFR	1.20	130	0.04	62.6

Why the differences in the areal algal production occurred?

Investigations on algal growth and TSS concentrations in both reactors were conducted to find the reason.

- The apparent algal growth rate was controlled by the physical and chemical growth conditions, such as light intensity, nutrient concentration, and the SRT, but it was not affected by the HRT or reactor volume. Because as shown in Equations 3.7 and 3.8, the apparent growth rate is not a function of HRT or reactor volume. When the physical and chemical growth conditions and the SRT are the same and consequently the apparent growth rate is the same, the algal masses of the SBR and CFR should be the same under the steady state.

- Interestingly our present study shows a significant difference in algal concentration and algal production rates. This result might be attributed to the different HRT operation among the reactors due to the availability of sufficient nutrients supply. The daily flow rate of greywater in SBRs was twice as much as that of CFRs. Obviously this has affected the TSS concentrations in both reactors: the algal concentration in SBRs (HRT= 10 days) was higher than that of in CFRs (HRT = 20 days). Similar observation was as well noticed for the algal productivity per liter of influent (Table 3.4).

  Further, due to the high algal concentration found in SBRs and due to the daily withdrawal of excess algae from these reactors, the algal productivity became two to three times higher (Table 3.4) than that of CFRs. In contrast the no excess algae removal in CFRs may result in lower algal production rates. In both reactors the limitation of algal growth due to insufficient nutrients was avoided.

  Results from our present study imply that operating a CFR with a volume two times larger than that of SBR and with a similar influent flow rate as SBR would result to the production of half of algal biomass amount produced in SBR. Additionally, by simulating the HRAP under continuous operation or batch operation with algal recirculation, the control of the algal productivity could be achieved.

3.4.2. Nutrient removal

The negligible nitrate concentrations and low pH observed in both reactors confirm that nitrification and ammonia volatilization were not the major processes responsible for nitrogen removal. The short HRT and the effect of sedimentation applied in the SBRs resulted in a higher algal concentration (Figure 3.2) and thus promoted nitrogen removal by assimilation into the algal biomass and the sedimentation process, similarly to that observed in the HRAP. In contrast to SBRs, low $NH4^{+}$-N uptake has occurred in CFRs where the algal concentration was lower (Figure 3.2). As a result, higher $NH4^{+}$-N concentration was found in E-CFRs (Table 3.3).

Since the greywater did not contain enough cations ($Fe^{2+,}$ Al^{3+}, Ca^{2+}, Mg^{2+},…), inorganic phosphate might not be removed by the precipitation and adsorption processes in both reactors (Diaz et al., 1994; Nurdogan and Oswald, 1995). In these reactors, phosphorus might have been removed through assimilation/sedimentation or through a combination of growth and the uptake of phosphorus (Powell 2009). By comparing N and P elimination, much less phosphorus than nitrogen was removed from the reactors during the monitored experimental period. The N:P ratio of algae was 15:1 (Redfield, 1934) and consequently, the greywater with a N:P ratio of 4:1 did not contain sufficient nitrogen to enable the complete removal of phosphorus by the assimilation process (Craggs, 2010; Nurdogan and Oswald, 1995).

3.5. Conclusions

In this study, a series of batch experiments were carried out to simulate the high-rate algal pond (HRAP) with algae recirculation and a series of continuous experiments reproduced the operation of usual HRAP without algal recirculation.

- In contrast to CFR, the operation of HRAP under batch mode has enhanced the selection of settleable algae through the sedimentation process.
- In SBRs and CFRs, because of the same SRT of 20 days, the algal growth rate was similar. However, the high algal productivity observed in the SBRs was attributed to the short HRT.
- The nutrient concentrations were higher in CFRs than in the SBRs and the biomass uptake was the main mechanism responsible for the nutrient removal.
- By simulating the HRAP under continuous operation or batch operation with algal recirculation, the control of the algal productivity and the independent control of HRT and SRT were achieved.

Chapter 4 : Operation of a high rate algal pond for greywater treatment and the reuse of resources

4.1. Introduction

This chapter focuses mainly on the production of resources from the high rate algal pond (HRAP) to serve the agriculture in arid and semi-arid areas. Moreover, for the implementation of high rate algal pond (HRAP) and the reuse of natural resources from the greywater treatment, operating strategies of HRAP in urban areas of arid and semi-arid countries were indicated.

Experiments were achieved by using the systems described in Chapters 2 and 3. Thereby, in HRAP, SBRs and CFRs, nitrogen and phosphorus balance were assessed. Furthermore, from the operating condition of each type of reactors, the ability of HRAP to enhance algae production and the recovery of resources by varying the hydraulic retention time (HRT) and solid retention time (SRT) was mentioned.

4.2. Materials and methods

4.2.1. Operation conditions and analytical methods in HRAP, SBRs and CFRs

Table 4.1 summarizes the operating conditions in HRAP, SBRs and CFRs as indicated in Chapters 2 and 3. These reactors were monitored during two phases as reviewed in Table 4.1. In phase 1 the effect of the HRT was evaluated by monitoring three replications of a sequencing batch reactor (SBR) and three replications of a continuous flow reactor (CFR). In phase 2, the effect of the SRT was assessed by monitoring an HRAP reactor system.

Table 4.1. Operation phases

Phases	Reactors	HRT	SRT	Replications	Investigations
1	SBR	10	20	3	Effect of HRT
	CFR	20	20	3	
2	HRAP	8	10, 15, 20	1	Effect of SRT

The same synthetic greywater indicated in previous chapters was used in all reactors (Chapters 2 and 3).

The setup of HRAP was same as described in Chapter 2 (Figure 2.1). Operating modes and control of SRT were also same as indicated in Chapters 2. Experiments in SBRs and CFRs were performed as indicated in Chapter 3 (Figure 3.1) with similar operating conditions.

Temperature and pH, total suspended solids and all nitrogen and phosphorus species were measured and determined as explained in Chapter 2 and 3.

4.2.2. Total algal biomass productivity in reactors

The total algal productivity of each reactor was calculated using Equations 4.1 and 4.2.

Equation 4.1. Total algal biomass productivity of SBRs and HRAP per liter of influent

$$P\ (mg/L) = \left(X_{\text{Excess algae}} + X_{Effluent} + \Delta X_R\right) / Influent_{flowrate}$$

P : Total productivity of SBR or HRAP (mg/L)

$X_{\text{Excess algae}}$: Algal biomass withdrawn from the SBR (mg/d)

$X_{Effluent}$: Algal biomass in effluent from SBR or HRAP (mg/d)

ΔX_R : Variation of algal biomass in SBR or HRAP (mg/d)

$Influent_{flowrate}$: Influent flow rate (L/d)

Equation 4.2. Total algal biomass productivity of CFRs

$$P_{\mathrm{CFR}}(mg/L) = \left(X_{\mathrm{E\_CFR}} + \Delta X_{\mathrm{CFR}}\right) / \, Influent_{flowrate}$$

P_{CFR} : Total productivity of CFR (mg/L)

$X_{\mathrm{E\_CFR}}$: Algal biomass in effluent from CFR (mg/d)

ΔX_{CFR} : Variation of algal biomass in CFR (mg/d)

4.2.3. Nitrogen and phosphorus mass balance calculation

The rates of organic nitrogen, ammonium-nitrogen, nitrate and nitrite-nitrogen, soluble phosphorus and total phosphorus were estimated during the period of steady state.

- The nutrients fraction in the water leaving the SBRs includes: the fractions of the dissolved and particulate samples of the SBR's effluents and excess algae. The Equation 4.3 below describes how the total amount of nutrients exiting the SBRs was assessed:

Equation 4.3. Nutrients fractions in the effluent and the excess algae from SBRs

$$Total\,exiting_{SBR}(mg/d) = Dissolved_{E-SBR} + Dissolved_{excess\,algae} + Particulate_{E-SBR} + Particulate_{excess\,algae}$$

$Total\,exiting_{SBR}$: Total nutrients fractions leaving the SBR (mg/d)

$Dissolved_{E-SBR}$: Fraction of nutrients in dissolved samples of the effluents from SBRs (mg/d)

$Dissolved_{excess\,algae}$: Fraction of nutrients in dissolved samples of the excess algae (mg/d)

$Particulate_{E-SBR}$: Fraction of nutrients in particulate samples of effluents from SBRs (mg/d)

$Particulate_{excess\,algae}$: Fraction of nutrients in particulate samples of excess algae (mg/d)

- The nutrients fraction in the water leaving the CFRs includes: the fractions of the dissolved and particulate samples of the CFR's effluents. The equation 4.4 below describes how the total amount of nutrients exiting the CFRs was assessed:

Equation 4.4. Assessment of the nutrients concentration in the effluent from CFRs

$$Total\,exiting_{CFR}(mg/d) = Dissolved_{E-CFR} + Particulate_{E-CFR}$$

$Total\,exiting_{CFR}$: Total nutrients fractions leaving the SBR (mg/d)

$Dissolved_{E-CFR}$: Fraction of nutrients in dissolved samples of the effluents from CFRs (mg/d)

$Particulate_{E-CFR}$: Fraction of nutrients in particulate samples of effluents from CFRs (mg/d)

4.3. Results and discussion

4.3.1. Nitrogen balance

- High rate algal pond (HRAP)

Figure 4.1 presents the different amount of total nitrogen (T-N) entering and leaving the HRAP system. The results showed that the operation of shorter SRT in the system has enhanced the removal rate of TN in the effluent. In fact, this state might be relying on the performance of the algal productivity in the reactor. Furthermore, the rate of denitrification that occurs could be also assessed. For the different SRT of 10, 15 and 20 days, very small denitrification rate was assessed in the reactor (Figure 4.1). In fact, since the pH in the reactor was less than 8.5, volatilization of ammonia through the pond surface was limited (Su et al., 2012) . Ultimately, the assimilation of the nitrogen by the algal biomass and the sedimentation processes has occurred in the reactor and the ASP. In this regards, as mentioned Lai and Lam (1997) these two processes were proposed as the main mechanisms responsible of the nitrogen removal.

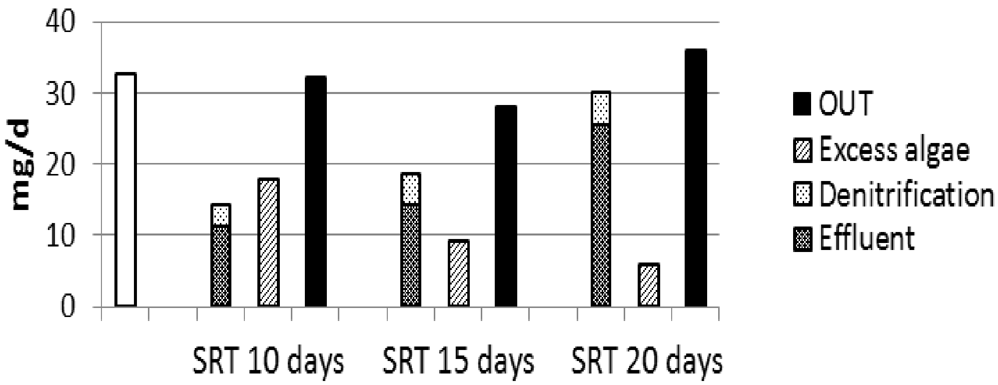
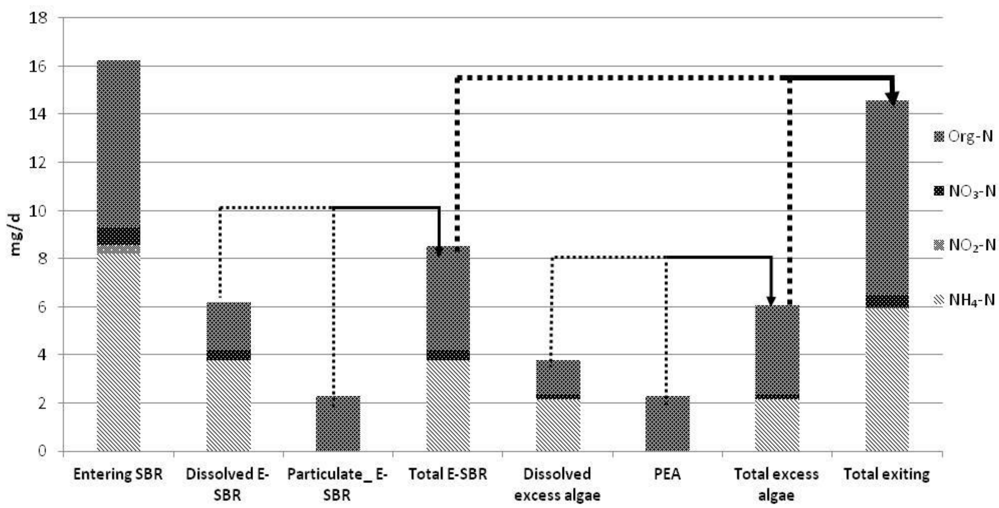


Figure 4.1. Total Nitrogen balance in HRAP

- Sequencing batch reactors (SBRs) and continuous flow reactors (CFRs)

The current nitrogen balance was established during the period when the algal growth in the reactors was not subject to change. From the SBRs, the rate of nitrogen exiting the system was assessed by the daily washout of excess algae added to the overflow of the treated effluent. In this study, the influent used for the SBRs and CFRs was composed by a large part of ammonium nitrogen and organic nitrogen (about 8 and 7 mg/d respectively for the SBRs and half of these amounts for the CFRs). The nitrate and nitrite concentrations represented only about 1 mg/d for the SBRs and 0.5 mg/d for the CFRs. Additionally, for reactors, ammonium-nitrogen and organic nitrogen were removed (Figures 4.2.a and 4.2.b). Contrary to the CFRs where the reactor's mixture continuously overflowed, the contribution of the excess algae withdraw from the SBRs has led to the increase of the nitrogen exiting this system.

a)



b)

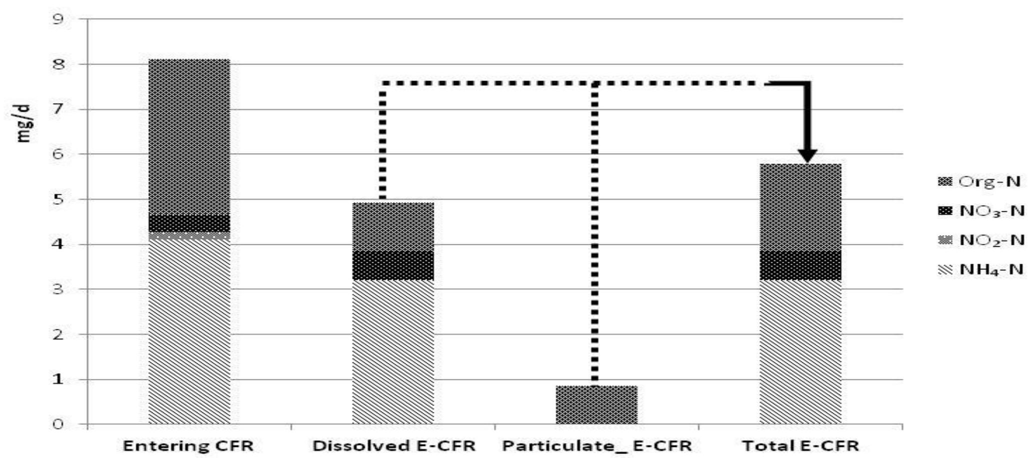


Figure 4.2 . Nitrogen species concentrations in the influent and the effluent from the SBRs and CFRs. (a) SBRs; (b) CFRs

4.3.2. Phosphorus balance

- High rate algal pond (HRAP)

The assessment of the phosphorus fraction in the effluent (Figure 4.3), suggests that a weak elimination of the phosphorus from the HRAP has occurred. This state was explained by Redfield (1934) who noticed that algae had N:P ratio of approximately 15:1. Consequently, the wastewater N:P ratio (about 4:1) did not contain sufficient nitrogen to enable the complete phosphorus removal by assimilation process (Craggs, 2010). In the reactor, the phosphorus uptake by assimilation/sedimentation depended mainly on the algal biomass concentration (El Hamouri, 2009).

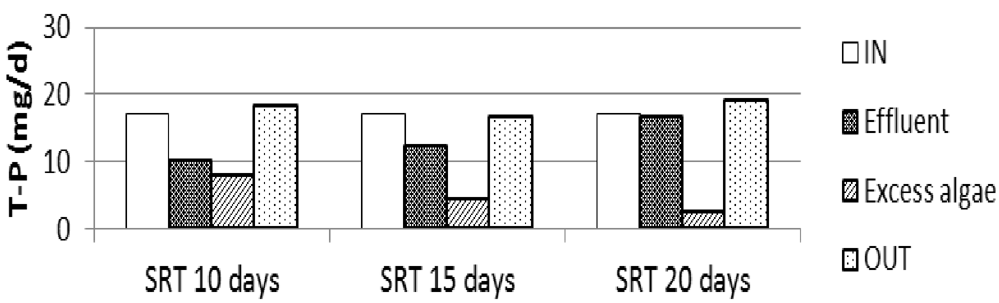
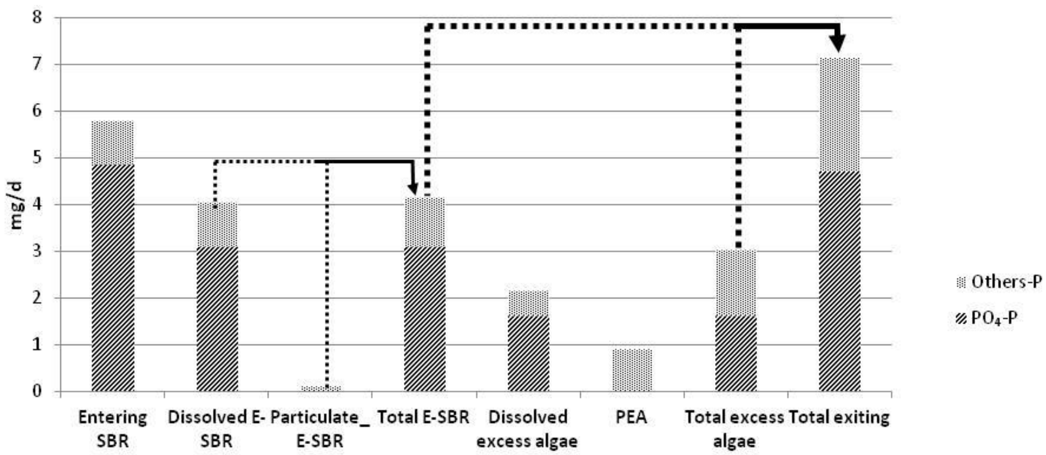


Figure 4.3. Total phosphorus balance in HRAP

- Sequencing batch reactors (SBRs) and continuous flow reactors (CFRs)

The results presented in Figures 4.4.a and 4.4.b showed that, from the SBRs, the contribution of the excess algae and the fraction of organic phosphorus could justify the high phosphorus amount exiting these reactors.

a)



b)

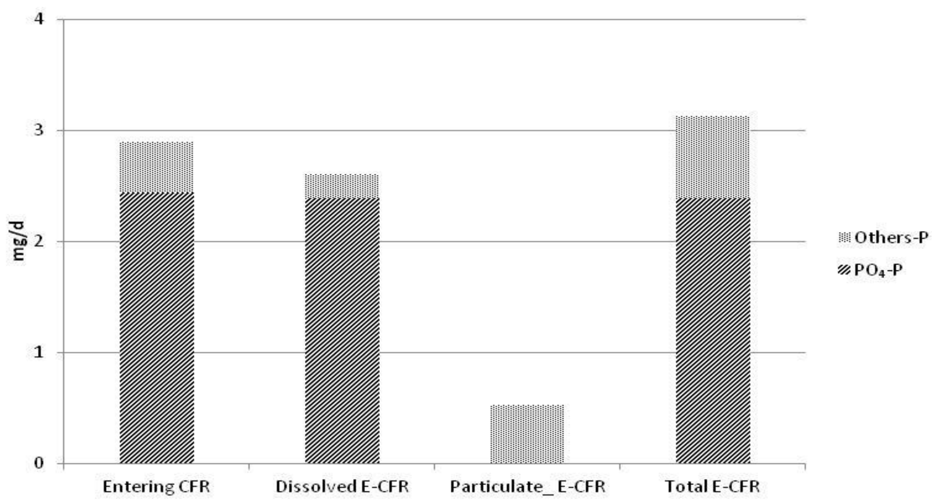


Figure 4.4. Phosphorus species concentrations and balance in SBRs and CFRs systems. (a). SBRs; (b). CFRs

4.3.3. Effect of the operating parameters

Table 4.2 shows the different tendencies of algal biomass productivity and algal and nutrients removal when various HRTs and SRTs were employed. Thereby, the influence of employing short and long HRTs in the SBRs and CFRs was investigated, and the impact of controlling the SRT in the HRAP was also considered.

- Potential effects of the HRT and SRT on algae productivity

Higher algal biomass productivity was found when a short HRT of 10 days coupled with an SRT of 20 days was employed, whereas a long HRT of 20 days coupled with an SRT of 20 days resulted in lower algal productivity (Table 4.2.a). During the short HRT of 10 days in the SBRs, the algae productivity was assessed using Equation 4.1 and includes the biomass of the excess algae. In the SBRs, owing to the negligible proportion of the algal biomass of the effluent, the algae productivity was dependent on the biomass of excess algae. On the other hand, when a long HRT of 20 days coupled with an SRT of 20 days was employed, the biomass productivity depended on the algal biomass of the effluent (Equation 4.2) owing to the nonexistence of excess algae. By operating HRT<SRT, the algal productivity depended on the excess algae and was increased.

The effect of the SRT on the algal biomass confirmed that with increasing SRT, biomass production gradually decreased and, in contrast, a shorter SRT increased biomass production (Table 4.2.a). Considering that the algae productivity was assessed through Equation 4.1, various facts could explain this difference:

- The algal concentration in the excess algae was higher than that the in effluent
- When a long SRT was employed, the volume of excess algae decreased. Consequently the algal productivity, which depended mainly on the biomass of excess algae, also decreased.

- Reduction and maximization of excess algae concentration

In both reactors the excess algae represented the daily washout of the reactor’s mixture. For the operation with short and long HRTs, the results in Table 4.2.b show that the concentration of excess algae was high when an HRT of 10 days coupled with an SRT of 20 days was employed in the SBRs. Moreover, when operating the CFRs with an HRT of 20 days coupled with an SRT of 20 days in CFRs, excess algae was not generated. In the CFRs, where continuous operations were performed, this deficiency occurred as a result of the nonexistence of any form of solid retention. The detention times of the liquid and the algal biomass were the same in the reactor (von Sperling, 2008).

In the HRAP, with increasing SRT, the algal concentration of the mixture washout gradually increased, similarly to the algal concentration of the mixed liquor. This increase in the algal concentration during the long SRT of 20 days might have been due to the long period of storage of the algae in the reactor.

- TSS effluent and nutrient concentrations

The algal concentration of the effluent was lower for a short HRT and a long SRT (Table 4.2.c), implying that settleable algae was dominant when an HRT of 10 days was employed in the SBRs and when an SRT of 10 days was employed in the HRAP (Figure 4.5). In parallel, the measured values of dissolved total nitrogen (D-TN) and dissolved total phosphorus (D-TP) showed greater removal efficiencies under the same operating conditions (Table 4.2.d). These results confirmed the removal of nitrogen and phosphorus through the assimilation processes that occurred in both reactors.

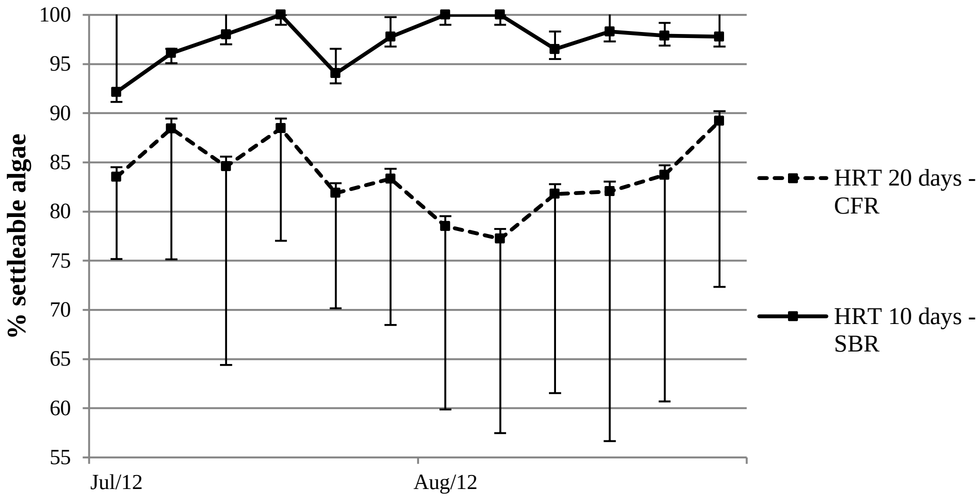
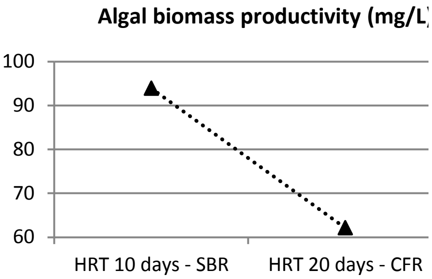
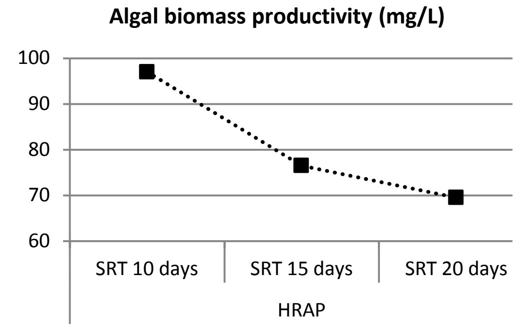
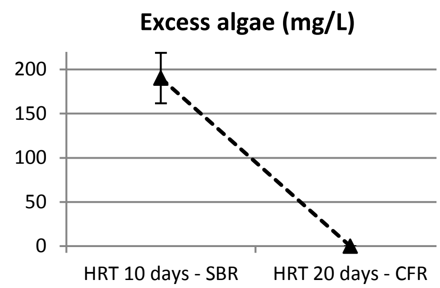
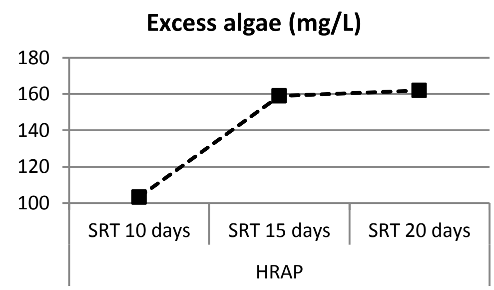
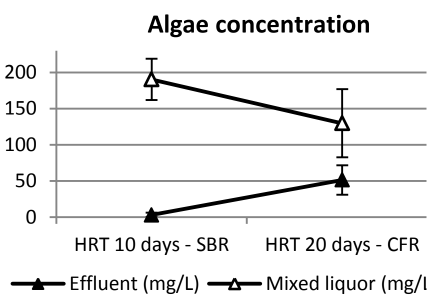
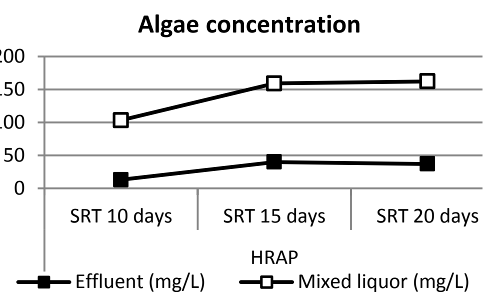
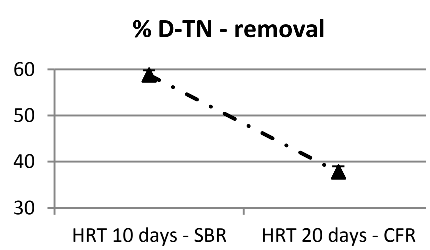
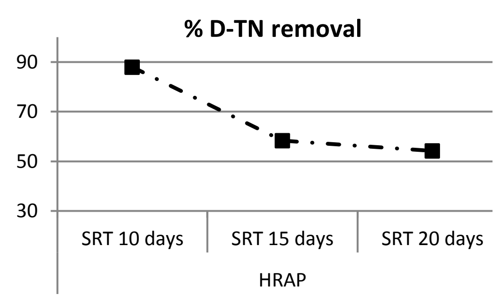
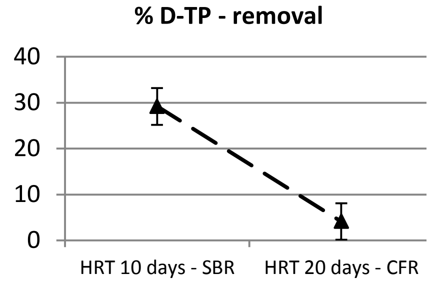


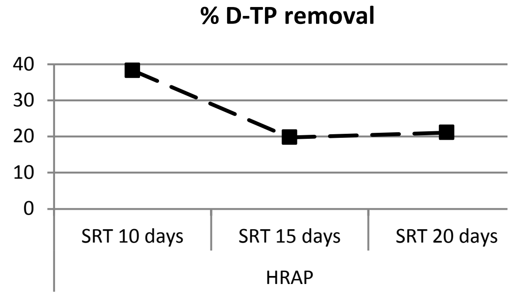
Figure: 4.5 . % of settleable algae during operations with HRT of 20 days and 10 days

Table 4.2 . Effects of the HRT and SRT on algal biomass productivity, algal biomass removal, and effluent quality

	HRT	**SRT control**
Algal biomass production (L^{-1} of influent)		
Excess algae		
Algae concentration		
Dissolved nitrogen and dissolved phosphorus removal		

	HRT	**SRT control**





4.3.1. Options for the reuse of resources and economic considerations

Table 4.3 summarizes the treated water quality obtained in this study. For both operating modes, TSS concentration in the effluent was almost equal to the 50mg/L (the target of treatment), except when SRT 10 days was controlled. Results of nutrient concentrations obtained for each set of operation have suggested that in all cases, the treated effluent contains sufficient nitrogen and phosphorus for the plant growth.

Table 4.3. Selection of operating parameters for the reuse of treated effluent and biomass

Parameters	Target of treatment	Treated water quality (Average concentrations)		
		SRT 20 days – HRT 8 days (HRAP with algal recirculation)	*SRT 20 days - HRT 20 days (conventional HRAP without algal recirculation)*	*SRT 10days – HRT 8 days (HRAP with algal recirculation)*
TSS (mg/L)	<50	37	51	13
T-N (mg/L)		5	9	2
T-P (mg/L)		5	5	3

The effluents and algal biomass collected from the different reactors exhibited variability in their nutrient contents. Table 4.4 summarizes the potential of each set of operation parameters for the reuse of the effluent and biomass.

Among all the applications based on water reuse, the disposal and storage of effluent in reservoirs have become an integral part of the valorization of treated effluent. An issue involved in applications based on water reuse concerns the water quality discharge requirements. By employing a short HRT of 10 days or a short SRT of 10 days, nutrient removal was maximized (Table 3.d). Although the temperature and pH were reported to be important growth parameters (Abu-Rezq et al., 1999; Christenson and Sims, 2011), in all the experiments in SBRs, CFRs, and HRAP, these parameters had no impact on the performance of reactors. Thereby, in both reactors, the measured values of pH were 7.5 – 8.0 and the temperature was maintained at 30 °C.

As agricultural activities are developing in most cities in arid and semi-arid areas, it is often difficult to find clean water sources. Thereby, the use of treated greywater can be considered as an alternative to crop irrigation, depending on the inorganic matter, nitrogen, phosphorus and potassium contents. As shown in Table 4.3, the treated water resulting from a long HRT of 20 days or a long SRT of 20 days contains significant amounts of T-N and T-P for food crop production, thus reducing the need for chemical fertilizers.

Since algae biomass contains nutrients such as nitrogen, phosphorus, and potassium, it is considered to be an excellent fertilizer (Metting,et al, 1988). As indicated previously, by employing a short HRT of 10 days or a short SRT of 10 days, greater algal productivity was observed (Table 3.a). Under these conditions, the algal biomass assimilated the nutrients from the wastewater and D-TN was more effectively removed than D-TP (Table 4.2.d).

In the context of small communities with limited resources and untrained operating personnel, the implementation of HRAPs has been proposed owing to their low energy requirement (Oswald, 1991). Recently, considerable attention has been focused on the use of HRAPs for the production of algal biomass as a basis for biofuel production or animal feed (McCann, 2011). In addition, HRAPs can be designed to increase water gain (McCann, 2011) and to recover algal biomass as a fertilizer (Metting, Rayburn, and Reynaud, 1988; Craggs, 2010a).

Table 4.4. Selection of operating parameters for the reuse of treated effluent and biomass

Operating parameters	Biomass reuse	
	Irrigation	*Fertilizer*
Long HRT of 20 days	High potential	-
Short HRT of 10 days	-	High potential
Long SRT of 20 days	High potential	-
Short SRT of 10 days	-	High potential

(a) The treated effluent quality is based on the nitrogen and phosphorus concentrations.

4.4. Conclusion

The lab scale experiments demonstrated that:

- The contribution of the daily withdrawal of the excess algae and the operation of algae recirculation has increased the fraction of N and P leaving the reactors
- Operations with a short HRT of 10 days or a short SRT of 10 days resulted in higher algal biomass productivity than that with a long HRT of 20 days or a long SRT of 20 days.

For urban agricultural irrigation,

- Operating HRAP with algal recirculation and short SRT of 10 days would be better for the use of reclaimed greywater in irrigation
- Depending on the method of irrigation, long HRT and long SRT operation might be recommended to meet the different needs.

Chapter 5 : *Escherichia coli* and coliphages MS2 and Qβ removal in a high rate algal pond treating greywater

5.1. Introduction

In arid and semi-arid areas the reuse of the greywater for irrigation purpose presents many challenges including the risk of pathogen infection. Especially in developing countries where water for irrigation is lacking and inadequate, effective and low-cost disinfection system must be implemented. As others conventional ponds, the high rate algal pond (HRAP) offers low-cost and effective disinfection appropriate for use in developing countries (Mara, 2004 and Maynard et al., 1999). In HRAP, algae play an essential role in the process of pathogen removal by raising the pH and dissolved oxygen concentration which favor inactivation of bacteria. In high rate algal ponds mechanisms, of pathogen removal for improving the disinfection are needed. While several studies have concluded that sunlight represents the major process for pathogens removal (Leduc and Gher, 1990 and Maynard et al., 1999) other secondary factors such as temperature (Mara et al.,1992), pH and dissolved oxygen (Curtis et al., 1992) have also been investigated. Despite the significant efforts, there is still little information about the disinfection processes in HRAP as well as their relative importance.

The main objective in this chapter was to investigate disinfection processes occurring in HRAP. Therefore under tropical climate, series of batch experiments were set up to evaluate the potential of algal sedimentation and others pathways of *E. coli* and coli phages (MS2 and Qβ) inactivation.

5.2. The health based target of disinfection in HRAP

The target of disinfection depends on the risk scenario, initial concentration of pathogen in greywater and dose-response relationship. In this study, the quantitative microbial risk assessment (QMRA) in WHO guidelines (WHO, 2006b) was applied to set the target of disinfection in HRAP. According to the QMRA (WHO, 2006) rotavirus is the critical pathogen for unrestricted irrigation. The number of rotavirus in greywater is calculated by below equation.

$$\frac{0.04[\text{g per person per day}] \times \text{excretion density [numbers/g feces]} \times \text{excretion time [days]} \times \text{yearly incident}}{64{,}900\ [\text{ml/day}] \times 365\ [\text{days}]}$$

The excretion density of rotavirus is 10^7-10^{11} and yearly incident was assumed as 0.05, the number of rotavirus in greywater is $3x10^{-1}$ to $3x10^4$ /mL. Since the number of rotavirus in wastewater containing feces is 10^{-1} to 10^2/mL (WHO, 2006b), Table 5.1 , estimated number of $3x10^4$ in greywater is too many. $5x10^0$/ml in greywater was chosen as it might be the reasonable number.

The ingestion scenario is that 100g of lettuce is consumed per person every two days throughout a year and 10 mL of treated greywater remains on 100g lettuce after irrigation. WHO usually sets disability adjusted life years (DALYs) as 10^{-6}, while this risk is associated with mild diarrhea (e.g. with a case fatality rate of $1x10^{-5}$) at an annual disease risk of 10^{-3} (WHO, 2006a). From the calculation of QMRA, the dose of rotavirus becomes $5x10^{-5}$ per exposure event. From the ingestion scenario, rotavirus concentration should be $5x10^{-6}$/mL in the treated greywater for irrigation of lettuce. The required reduction for raw greywater is 6 log units. Since the decrease of viral numbers after last irrigation is estimated 2 to 4 log units, the required reduction in HRAP is 4 log units. The monitoring of viral numbers is difficult, so the monitoring level of E. coli corresponding to $5X10^{-6}$/mL of rotavirus is set 10^3/100mL (WHO, 2006b) and the E. coli numbers in greywater is 10^6/100mL. The summary of disinfection target is shown in Figure 5.1. The numbers of rotavirus is altered to the number of MS2 or Qβ in this study.

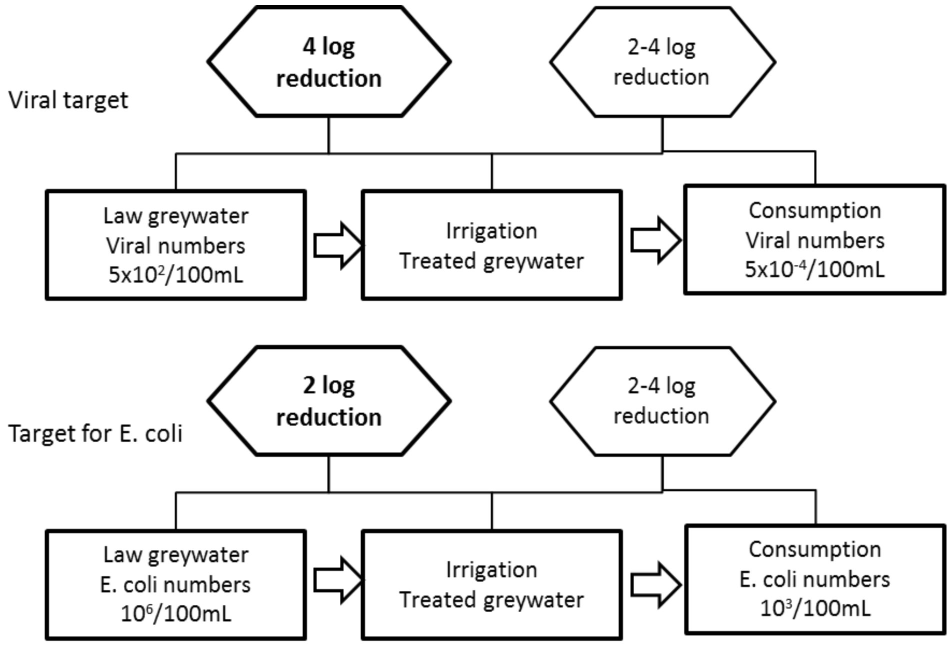


Figure 5.1. Health based targets of disinfection in HRAP

5.3. Material and methods

5.3.1. Disinfection experiment

Batch experiments were designed to determine the effects of sunlight, algal mixture and natural decay; for each environmental variable separate experiment were set up. *E. coli* (NBRC 3972) and coli phage MS2 and Qβ were used as indicators of pathogenic bacteria and virus.

Disinfection in HRAP was simulated as shown in Figure 5.2. The mixture of algae and bacteria was taken from the bench scale HRAP reactor (Figure 2.1.). Concentration of SS was adjusted using filtrate of effluent from HRAP. 0, 50, 100, 150, 200 and 250 mg/L of adjusted SS concentrations were applied in the experiment.

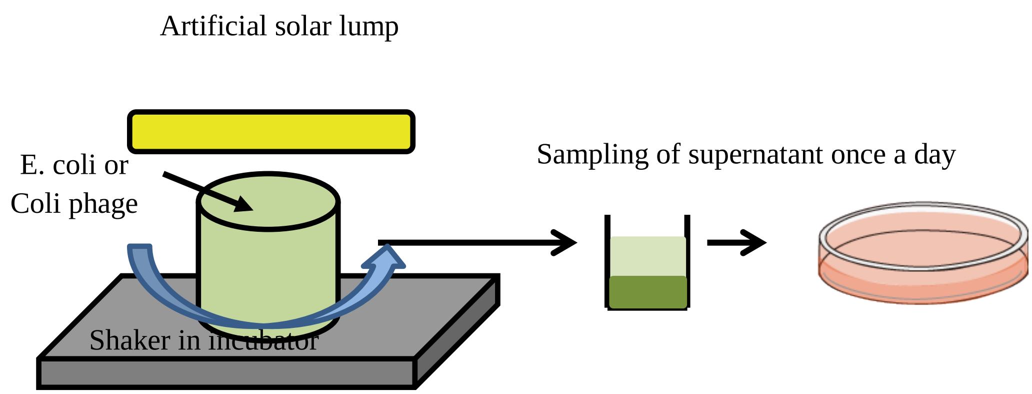


Figure 5.2. Experimental procedure of disinfection in HARP

One liter beaker was filled with algal mixture with depth of 5 cm and set to the shaker. The beaker was spiked with *E. coli*, MS2 or Qβ at the initial concentrations shown in Table 5.1.

Table 5.1. Initial concentration of pathogen indicators in reactor

Microorganism	**E. coli**	**MS2**	**Qβ**
Initial Number	$4.0x10^4$ CFU/ml	4.0x106 PFU/ml	$1.5x10^7$ PFU/ml

Then the shaker was installed in an incubator of 30 degree Celsius and shaken at the speed of 125 per minute. No light came from outside of incubator but an artificial solar lamp (XC-100, 100w, Seric Co. LTD.) was installed in the incubator. The UVA irradiation was kept at 29 w/m^2,which corresponded to the average UVA irradiation of daytime in Ouagadougou, Burkina Faso.22) UV irradiation was measured by UVA (wave length 315-380nm) meter (TM-208, Tenmars electronic Co. LTD.). At the UVA irradiation case, throughout the experimental period, the lamp was turned on from 6 AM to 6 PM and at dark case, the lamp was turned off. The experiment was continued for eight days, and artificial greywater shown in Table 5.2 was added to the beaker once a day to keep the activity of algal mixture.

Table 5.2. Composition of artificial greywater in reactor

Substrate	**Concentration mg/l**
Dextrin	9.2
Poly-peptone	20
Ichthyic extract	6.72
Yeast extract	20
KCl	4
NaCl	2
$MgSO_4$	2.5
KH_2PO_4	21.79
NH_4Cl	27.95
KNO_3	14.22
Ferric citrate	6

5.3.2. Microorganism culture, sample collection and analysis

E. coli (NBRC 3972) and coli phage MS2 and Qβ were obtained from the NITE Biological research center (NBRC, Chiba, Japan).

The Difco^{TM} LB broth Lennox (BD Diagnostics, 2009) was used as a growth medium for *E. coli* culture. The culture flask was placed in a shaking incubator maintained at 37 °C. After 24 hours, suspended *E. coli* was collected by centrifugation (3000rpm, 15mn, 4 °C) and the suspensions were diluted with phosphate-buffer solution (PBS, pH 7.2). *E. coli* strain 13965 was used as host bacteria for MS2 and Qβ. The phage preparation and detection were performed as described by Shirasaki et al. (2009).

A part of algal mixture was taken from the reactor once days, then the algae were settled for a few minutes and 1 ml of the supernatant was sampled. The number of indicators in the supernatant was immediately analyzed. Chromogen X-Gal (Compact dry, Nissei Co. LTD.) was used for E. coli count and Plaque method (E. coli NBRC 13965) was applied for MS2 and Qβ count.

There are several approaches to express the effects of environmental variables. The simplest approach adopts additive effects of sunlight, algal mixture and natural decay (Equation 5.1 and

Table 5.3). Evaluation of inactivation was based on the inactivation coefficient K_T (Equation 5.1), and K_T was composed by UVA irradiation, removal associated by algal sedimentation or predation and natural decay. Each inactivation coefficient was expressed as K_1, K_2 and K_3.

Equation 5.1. Determination of the total inactivation coefficient

$$K_T = K_1 + K_2 + K_3$$

Where K_T is the total inactivation coefficient

The experiments consisted of three different cases as shown in Table 5.3.

Table 5.3. Experimental conditions of three cases and effective inactivation coefficients

Case	**Lighting**	**Algal mixture**	**Natural decay**	**Inactivation coefficient**
A	+	+	+	$K_1+K_2+K_3$
B	-	+	+	K_2+K_3
C	-	-	+	K_3

5.3.3. Data analysis

The different *E. coli* inactivation coefficients were estimated as shown in Equation 5.3:

Equation 5.2. *E. coli* inactivation coefficient

$$k = -\left(\log_{10} N_t / N_0\right) / t$$

Where k (d^{-1}) is the inactivation coefficient; N_t (CFU/mL) and N_0 (CFU/mL) are the final and initial numbers of *E. coli* per milliliter volume of water at day 0 and t respectively and t (d) represents the retention time.

5.4. Results and discussion

The example of inactivation at disinfection experiment was shown in Figure 5.3. The indicator was E. coli and inactivation effects were compared with/without algal mixture under lighting condition. At the first two days, the number of E. coli showed 1 log unit increase indicating the growth and recovery from the UV damage during dark condition. The inactivation coefficient K_T of the case with algal mixture was slightly higher than that of the case without it. In this experiment, the batch feeding of the substrate and E. coli caused the increasing of E. coli at the first day; hence this increase can be ignored in continuous flow reactor like HRAP. Based on the inactivation coefficient, both cases achieved more than 2 log units reduction within two days. Figure 5.4 shows the relationship between SS and inactivation coefficients of E. coli. The total inactivation coefficient K_T did not change so much between 0 to 250 mg/L of SS. The most influential factor was natural decay, and UVA irradiation affected only when SS concentrations were low. This result differed from the result of Davies-Colley et al., (1999) because they concluded that the irradiation of UV and visible light were the dominant factors for disinfection. Two reasons may explain this difference, one is continuance of experiment and another reason is difference of temperature. As for continuance, their reactor was irradiated by sun light for 7 or 8hours, and then their experiment was ended. On the other hand, our reactor was operated for 8 days including dark period when damaged DNA recovery of E. coli occurred. The recovery of E. coli might decrease irradiation effect in our experiment. The water temperature of reactor was controlled at 20 degree Celsius by Davies-Colley et al., (1999) on the other hand, our reactor was kept 30 degree Celsius. The high water temperature accelerated the natural decay of E. coli. The difference of strains may be another reason as their E. coli was natural strain and ours was established strain for the disinfection test. As expected, the effect of irradiation was diminishing in accordance with increase of SS concentration, the effect of adsorption to algal mixture and the predation was increasing to the contrary. However, the dominant factor was the natural decay when water temperature was 30 degree Celsius.

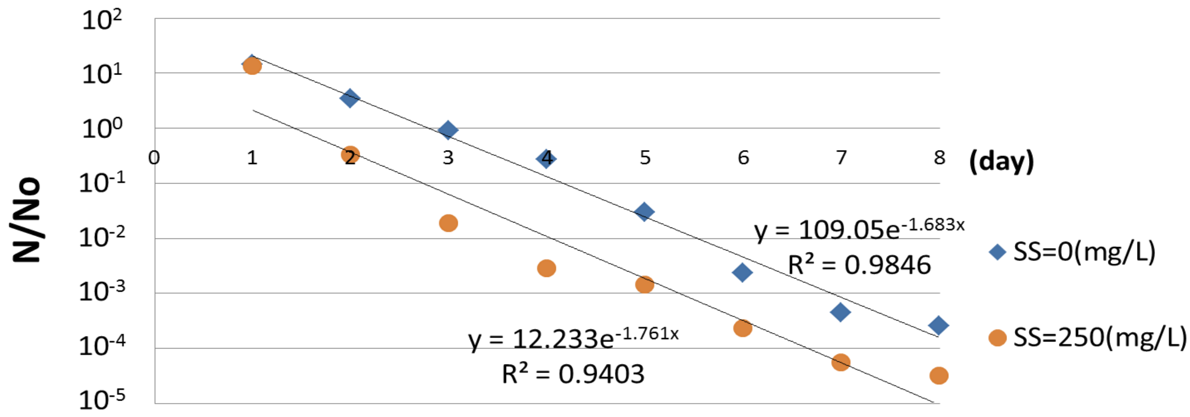


Figure 5.3. Inactivation of E. coli under sunlight lump with/without algal mixture

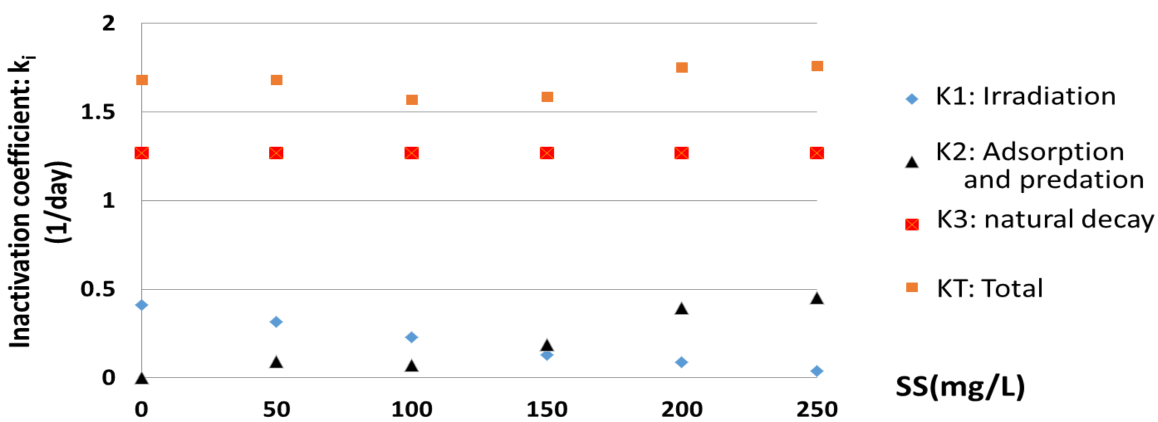


Figure 5.4. The relationship between SS and inactivation coefficients of *E. coli*

Inactivation of MS2 under lighting condition with/without SS is presented in Figure 5.5, showing slow inactivation speed compared with E. coli. The relationship between SS and inactivation coefficients of MS2 is shown in Figure 5.6. The irradiation was most effective factor and natural decay showed a little effect contrary to the result of E. coli. The result of Qβ was almost same as that of MS2 so it is skipped. Davies-Colley et al., (1999) monitored F specific RNA phage and F specific DNA phage in wastewater as viral indicators, and they found the reduction of larger than 1 log within 7 hours in their reactor and concluded that UV irradiation was exclusive factor for F-DNA phage. For F-RNA phage, irradiation of UV and visible light was main factors but other factors such as DO or SS affected disinfection. The reduction of MS2 in our experiment with SS was equal or less than 1 log unit during eight days. This difference might occur because of difference of species of virus.

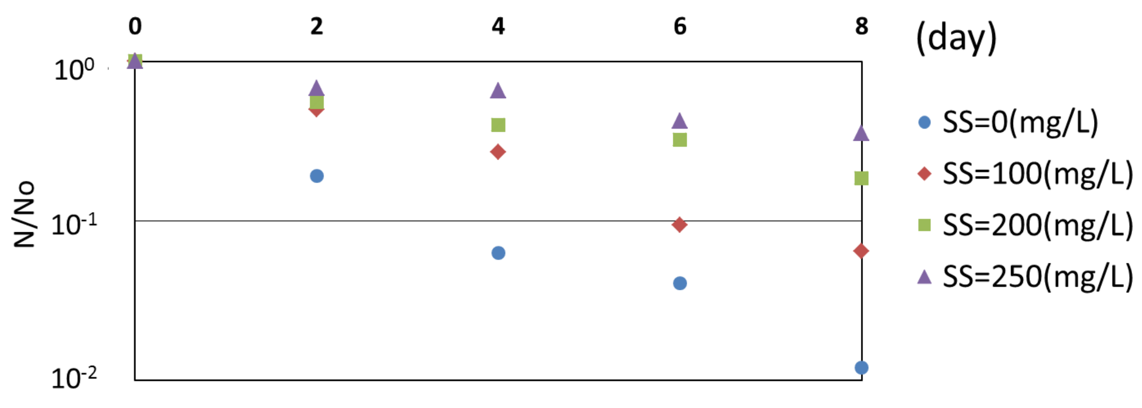


Figure 5.5. Inactivation of MS2 under sunlight lump with/without algal mixture

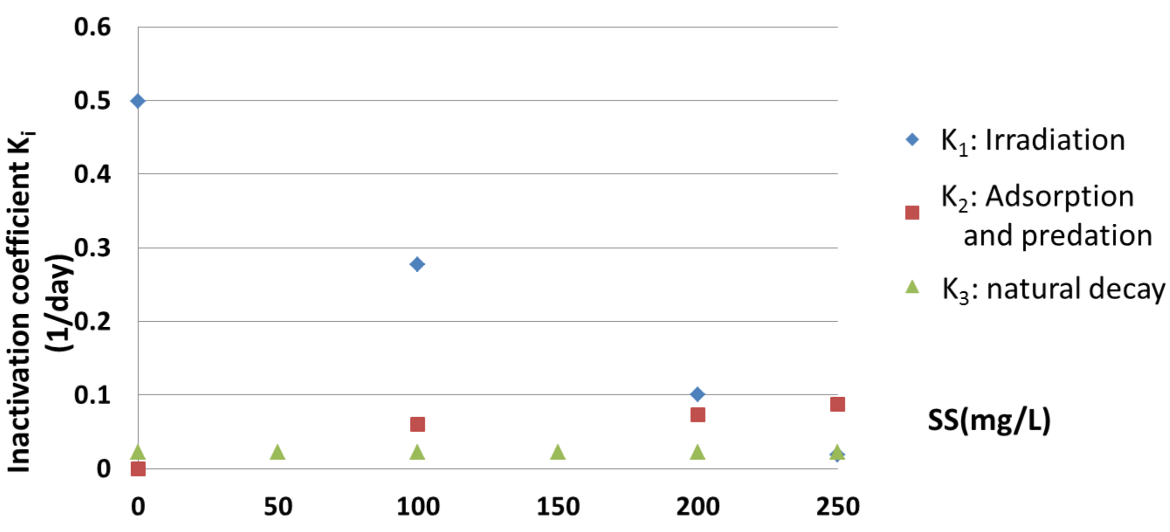


Figure 5.6. The relationship between SS and inactivation coefficients of MS2

The target of viral disinfection was set to 4 log units reduction thus it is impossible to attain the target under this operating condition. The pH of existing HRAP usually becomes larger than 9 in the daytime and the increase of pH affects disinfection effectively. However, pH in our reactor was around 7.5 throughout the experiment. Natural water is held in equilibrium with gaseous carbon dioxide in air. When activity of photosynthesis in HRAP becomes enhanced, the equilibrium condition moves to the left side of equilibrium equation then resulting of diminution of H^+:

$$Photosynthesis \leftarrow CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3$$

However, our reactor was shaken hard, 125/min, to confirm the complete mixing. Thus, enough gaseous CO_2 was provided to keep pH neutral. The pH in reactor was increased and adjusted to 9 during the experiment using 0.1M of NaOH. Figure 5.7 shows the inactivation of MS2 under pH control in dark condition. The reduction of 4 log units was observed within three days with no relation to SS concentration.

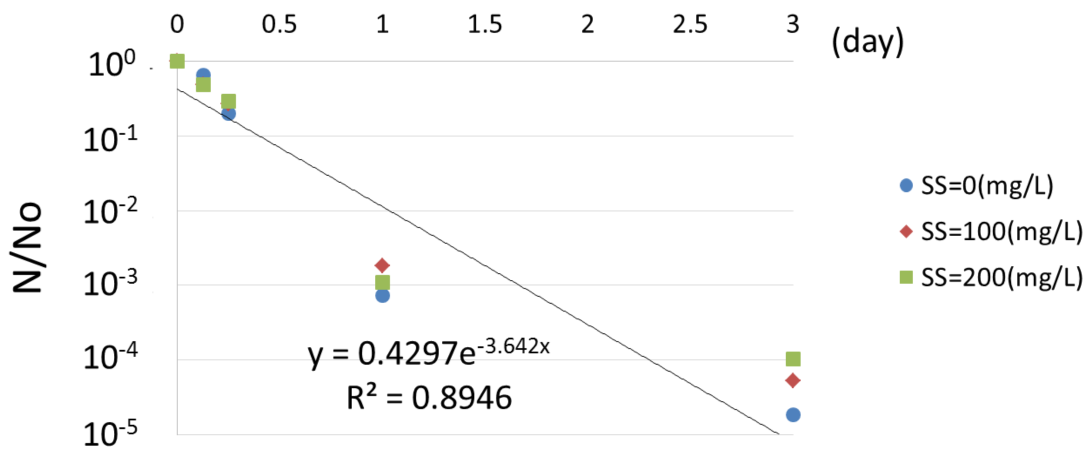


Figure 5.7. Inactivation of MS2 at pH 9 under dark condition with/without algal mixture

5.5. Conclusion

The targets of disinfection of greywater were set to 2 log unit reduction for E. coli and to 4 log units reduction for rotavirus when treated greywater was used for unrestricted irrigation. These targets depend on the number of pathogen in greywater; therefore the further monitoring of E. coli and the virus in greywater will be needed in developing country.

Water temperature in HRAP always exceeds 30 degree Celsius in tropical region, thus natural decay of bacteria was the dominant factor for bacterial disinfection. UV irradiation was effective, however the recovery of damage on DNA should be considered. The target for E. coli was achieved within HRT of two or three days.

Viral indicators of MS2 and Qβ were hard to reduce when pH was neutral. Irradiation was most effective factor for inactivation of MS2, but SS interfered with the penetration of light into the reactor. However, when pH was increased to 9, the reduction of 4 log units was observed within three days with no relation to SS concentration.

Chapter 6 : Conclusions and recommendations

This PhD study has mainly focused on the improvement of high rate algal pond (HRAP) to produce and harvest settleable algae, and to recover natural resources for agriculture practice in arid and semi-arid areas. The major conclusions from the studies can be summarized as follows:

- In the HRAP system, simultaneous algal recycling with the operation of variable solid retention time (SRT) has influenced the biomass bio-flocculation, and the algal and nutrients removal. By recirculating the algae, the dominant algal species could be maintained and higher settleability and removal efficiency of the algae could be achieved. Further, operating long SRT of 20 days has increased the TSS concentration in HRAP due the low TSS concentration of the mixture washout during long SRT. For operation under a short SRT of 10 days, efficient algal removal (86%) together with NH4 +-N and PO4 3--P removal (84% and 55% respectively) were achieved. In the algal settling pond (ASP), at SRT of 10 days, settleable algae were dominant and the selection has occurred by simple gravity sedimentation.
- Series of batch experiments were carried out to simulate the high-rate algal pond (HRAP) with algae recirculation and a series of continuous experiments reproduced the operation of conventional HRAP without algal recirculation. In contrast to CFR, the operation of HRAP under batch mode has enhanced the selection of settleable algae through the sedimentation process. In SBRs and CFRs, because of the same SRT of 20 days, the algal growth rate was similar. However, the high algal productivity observed in the SBRs was attributed to the short HRT. The nutrient removal efficiency was higher in the SBRs than in the CFRs, and the biomass uptake was the main mechanism that responsible for the nutrient removal. By simulating the HRAP under continuous operation or batch operation with algal recirculation, the control of the algal productivity and the independent control of HRT and SRT were achieved.
- In SBRs, where short SRT of 10 days was operated, algal productivity was (3.60 g/m2/d) higher than in CFRs where 1.20 g/m2/d was found during SRT of 20 days operation. The nitrogen and phosphorus balance have shown that the contribution of the excess algae withdrawn from the reactors has led to the increase of nitrogen exiting the system. On the other hand, the amount of phosphorus exiting both SBRs and CFRs was slightly higher than in the influent.

- Under tropical climate, series of batch experiments were set up to investigate the potential of algal sedimentation and others pathways of inactivation. It was found that the natural decay of bacteria was dominant and UV irradiation was effective. However the recovery of light-damaged cells at night should be considered.
- Operating strategies for HRAP application for the greywater treatment in urban areas of arid and semi-arid countries where defined. It was proposed that for urban agricultural irrigation, the selection of appropriate hydraulic conditions (long HRT and long SRT) can be implemented to meet different needs. In contrast, the operation of short HRT and short SRT provide effluent with a lower nutrient concentration which can be discharged in reservoirs. The recovered algal biomass from the HRAP could be used either as fertilizer or used as a livestock feed supplement. Focusing on conventional HRAPs, this method might be applied to achieve efficient productivity or removal of the algal biomass.

- For urban agricultural irrigation, operating HRAP with algal recirculation and short SRT of 10 days would be better for the use of reclaimed greywater in irrigation depending on the method of irrigation, long HRT and long SRT operation might be recommended to meet the different needs.

Besides the interest for biofuel and biogas production, future research effort should go towards investigating the application of HRAP as a sustainable sanitation technology option for urban areas in arid and semi-arid countries. To address current economic and technological issues of greywater management, considering the HRAP for a decentralized greywater treatment is a feasible option. This may contribute to an improve sanitation and promotes resources reuses for agricultural purposes. Due to the variable pollutants found in greywater and which result from daily activities, it might be beneficial to investigate on the ability of HRAP to remove such pollutants.

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