Article

Reduction in Energy Requirement and CO₂ Emission for Microalgae Oil Production Using Wastewater

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Received: 19 February 2020; Accepted: 25 March 2020; Published: 2 April 2020

Abstract: A comparative evaluation of energy requirement and CO₂ emission was performed for native polyculture microalgae oil production in a wastewater treatment plant (WWTP). The wastewater provided nutrients for algae growth. Datasets of microalgae oil production and their details were collected from the Minamisoma pilot plant. Environmental impact estimation from direct energy and material balance was analyzed using SimaPro[®] v8.0.4. in two scenarios: existing and algal scenarios. In the existing scenario, CO₂ emission sources were from wastewater treatment, sludge treatment, and import of crude oil. In the algal scenario, CO₂ emission with microalgae production was considered using wastewater treatment, CO₂ absorption from growing algae, and hydrothermal liquefaction (HTL) for extraction, along with the exclusion of exhausted CO₂ emission for growing algae and use of discharged heat for HTL. In these two scenarios, 1 m³ of wastewater was treated, and 2.17 MJ higher heating value (HHV) output was obtained. Consequently, 2.76 kg-CO₂ eq/m³-wastewater in the existing scenario and 1.59 kg-CO₂ eq/m³-wastewater in the algal scenario were calculated. In the HTL process, 21.5 MJ/m³-wastewater of the discharged heat energy was required in the algal scenario. Hence, the efficiency of the biocrude production system will surpass those of the WWTP and imported crude oil.

Keywords: biocrude; exhausted CO₂; oxygen ditch method; polyculture microalgae

1. Introduction

Energy security has become a concern in Japan since the accident of the Fukushima nuclear power plant. Furthermore, the global trend toward non-fossil fuels by the sustainable development goals (SDGs) is accelerating renewable energies. Hence, the microalgae biofuel production system is highly promising, but the challenge is its competitiveness with existing energy supply systems. Research has been conducted after the incident of the nuclear power plant to increase renewable energy production.

Microalgae is referred to as one of the best candidates for biofuel production, a source of renewable energy. Fuel production from microalgae in the Minamisoma pilot plant measuring 0.1 ha has been reported, which can facilitate 50 m³/day in an open raceway pond (ORP) [1]. Simulation was conducted based on experimental results that considered profitability, energy balance, and emission of environmental load (GHG). Major species of microalgae that had been cultivated were *Desmodesmus* sp., *Dictyosphaerium* sp., *Klebsormidium* sp., *Micractinium* sp., and *Scenedesmus* sp. [2].

The bottlenecks in profitability were nutrients and freshwater for algae growth. However, if wastewater were used for microalgae cultivation, costs could be reduced from 1605.9 JPY (14.1 USD: the exchange in Table A1 in Appendix A was used)/kg-biocrude-wet to 160.6 JPY (1.4 USD: Table A1)/kg-biocrude-wet [1]. Moreover, to fulfill energy demand, a bottleneck in energy balance was the energy requirement for circulating water in the ORP, the hydrothermal-liquefaction (HTL) process, and the centrifugation process.

A previous study showed that the use of wastewater with the addition of CO₂ gas was effective for microalgal growth and reduced CO₂ [3]. However, in that study, the introduction and the total system's emissions of wastewater treatment by microalgae were not explained because the details of the oil-production system from algae were unknown. The experiment at the Minamisoma plant showed that wastewater was a potential source from households that could be used after filtration in the ORP for microalgae production [4]. Wastewater could serve as a nutrient source for culturing microalgae, which could additionally reduce energy and CO₂. Theoretical calculations of microalgae oil production have been reported [5]; however, the theoretical and practical values calculated differed vastly. Hence, an accurate estimation of CO₂ emissions is highly required. A simulation is required based on the life cycle assessment (LCA) approach to project oil production potentiality from microalgae at the Minamisoma pilot plant.

Therefore, the purpose of this research is to evaluate the potential of microalgae oil production integrated with wastewater treatment including microalgae cultivation. Wastewater treatment and microalgae production could reduce the required energy and CO₂ emission, which could improve microalgae systems. This research differs from previous studies in the following two ways. First, the reproducibility in commercial plants is high because the parameters are set by experimental values from Minamisoma and not theoretical values. Secondly, the comparison is being made with the existing systems that are replaceable because of their superiority, with a thorough assessment of the applicability of the technology.

2. Methodology

The authors conducted repeated laboratory experiments, and field experiments using outdoor culture ponds of 33 m² which located in Kurihara (Ibaraki Prefecture), and culture pond of 1000 m² in Minamisoma. From the cultivation phase up to biocrude production phase, many issues were raised and a detailed procedure for full-scale plant design was obtained [4]. Based on these results, this study simulation was performed. The main reconstruction was emphasized based on five aspects: use of wastewater, reducing commercial nutrients, energy of circulation equipment, energy requirements for HTL, and centrifugation processes. These five aspects have been considered in a previous study regarding microalgae oil-production systems [5].

The suitable combination between an algae system and wastewater treatment plant (WWTP) was investigated; subsequently, an oxidation ditch (OD) method for WWTPs [6] was discovered. Regarding wastewater use, semi-continuous cultivation with overflowing wastewater (primary treatment water) and sodium acetate (0.3 g/L) in the first sedimentation basin was performed at the Tobu purification center (standard activated sludge method) located in the Ibaraki prefecture. Consequently, an algae biomass productivity of 0.037 to 0.049 g/L/day has been reported [4].

Algal production from primary treatment water has been confirmed. However, the possibility of raw sewage from the OD method can be used instead of overflowing wastewater from the standard activated sludge method (Tobu purification center). The auxiliary experiment, which was performed using raw sewage from the university's sewage pit, indicated a possibility. The result of algae productivity was 16.8 g/m²/day with a hydraulic retention time (HRT) of 4 days and add 5% CO₂ (1 L/min/10 L-wastewater) [7]. Therefore, algal production using raw sewage in the OD method was assumed, where wastewater can be used to reduce freshwater load. However, further research is required on algae productivity values.

Regarding the reduction of commercial nutrients, the waste from the HTL process could be recycled as nutrients [4]. The additional use of waste organic acids from the HTL process at a high algae concentration is effective for promoting growth. In that experiment, waste organic acids from the HTL process were treated by activated carbon. Half of the nutrients were recycled from the waste and the other half of the nutrients were provided by sodium acetate from commercial nutrients that were used in the initial stage of culture with low algae concentrations.

The energy requirement, such as circulation in an ORP, was calculated from existing facilities in Minamisoma [4,5]. However, in this study, the equipment size and number were optimized for the usage time and maximum daily processing capacity, of which the values were calculated using a function (Appendix A) that reflected the reality better. Also, energy consumption was reduced by changing the centrifugation to condensed sedimentation with flocculants. Furthermore, for the HTL energy, waste heat from power plants or other facilities was used for heat treatment, instead of electricity.

In terms of environmental impact, it was assumed that the exhaust gas and exhaust heat from the power plant could be used without any cost or environmental impact. Also, algae absorption in the culture pond was considered, as well as the emissions related to sludge cake treatment from the OD method. Therefore, two scenarios are proposed in this study: “existing wastewater treatment scenario (existing scenario)” and “algae integrated wastewater treatment scenario (algal scenario)”.

In the wastewater treatment system, the existing scenario begins with aeration and the algal scenario with cultivation (Figure 1). For comparison, both scenarios treat the same 1 m³ of wastewater and produce or import oil of 2.17 MJ. Furthermore, each process’ mass of 50-m³-based experiment was divided into 1 m³ (Table 1). Biocrude-wet was assumed as the final product in the algal scenario to compare with crude oil in the existing scenario. After the HTL process, the microalgae are converted to biocrude-wet and the aqueous phase. Subsequently, the substance that dries the biocrude-wet at a temperature of 105 °C until the weight change within 30 seconds is less than 0.01% is called biocrude-dry. The approximate value of two-third of the biocrude-wet to biocrude-dry (0.66) was obtained from the open raceway pond at Minamisoma (Dominant species: *Desmodesmus* sp.), under the university of Tsukuba [8]. One example, the ratio was 0.76, which calculated from below. The 798.8 g at 19.9% biomass suspended solid (SS) of biocrude-wet yielded 611.0 g of biocrude-dry. Under the experimental temperature of 350 °C and a pressure of 19.4 MPa in the HTL process. In another example, using a ratio of 0.69, a 669.7 g at 18.9% SS of biocrude-wet yielded 458.8 g of biocrude-dry. Under the experimental conditions of 350 °C and 19.5 MPa in the HTL process.

Figure 1. System boundary for existing and algal scenarios.

Table 1. Conversion ratio of microalgae to biocrude from the pilot plant located at Minamisoma.

Process	1-d Process		Biomass SS ¹ in Eject from Each Process (%)	HHV ² (MJ/kg)	Source
Microalgae					
Cultivation (ORP)	50 m ³	(1 m ³)	0.034	-	Literature [5]
Centrifugation	1441.00 kg	(28.802 kg)	1.156	-	Literature [5]
Drum filtration	70.81 kg	(1.415 kg)	20.000	-	Literature [5]
Extraction (HTL)	5.66 kg	(113.2 g)	Biocrude-wet	-	Literature [5]
Purification	-	(74.7 g *)	Biocrude-dry ³	29.1	Literature [4]
Crude oil	-	(48.4 g *)	-	44.9	Literature [9]

¹ Suspended solid, ² Higher heating value, ³ Conversion rate: 0.66, * Based on the same HHV.

Therefore, 113 g of biocrude-wet was purified to 74.7 g ($113.2 \text{ g} \times 0.66$) of biocrude-dry. Moreover, 74.7 g of biocrude-dry was equal to 48.4 g of crude oil, based on the same higher heating value (HHV) equivalent to 2.17 MJ ($0.0747 \text{ kg} \times 29.1 \text{ MJ/kg} \approx 0.0484 \text{ kg} \times 44.9 \text{ MJ/kg}$). The processes within the dotted lines represent the system boundary. However, input and output processes such as (O₂) and (CO₂) were not calculated.

2.1. Existing Wastewater Treatment Scenario (Existing Scenario)

The direct energy and materials for the existing scenario were calculated to estimate the environmental effect; 1 m³ of wastewater treatment was included in 690 g of the disposal sludge cake and 48.4 g of the imported crude oil. The construction energy and materials were not involved in this calculation because the constructed WWTP's ditch and equipment were likely to be used for algae production. All data to calculate the existing scenario were from a related study.

The existing scenario included CO₂ equivalent (eq) emissions for three operational units: emissions from the WWTP by the OD method, from the sludge cake disposal treatment, and from the imported crude oil. The OD method was selected so that the WWTP could be used as a competitor in the algal scenario. The treatment tank of the OD method was similar to the ORP for microalgae cultivation except for the water depth. The general water depth in the OD method was 2.5 m.

2.2. Algae Integrated Wastewater Treatment Scenario (Algal Scenario)

In the algal scenario, 1 m³ of wastewater can be treated by generating 113 g of biocrude-wet by cultivating the microalgae. The flocculation tank was set in this scenario because using a polymer flocculant and flocculation tank was more efficient than using centrifuges, which were used at Minamisoma (Table 2). If the centrifugal separator is used for dewatering, the energy usage will be 3–5 kWh/kg-dry-microalgae. Therefore, approximately 30 MJ/L-biocrude was required, which is comparable to the amount of heat generated by biocrude itself. Also, the flocculation system was already used in the OD method's WWTP in Japan. In addition, a technical methodology for wastewater treatment had been established and optimized.

Table 2. Evaluation of different methods in the primary dewatering process of microalgae production at Minamisoma [7].

Primarily Dewatering Process	Characteristic	Operating Cost	Adaptability
Centrifuges	Huge energy consumption (3.3 kWh/kg-dry-MA)	Energy cost is high	Existing at Minamisoma
Flocculation (Sedimentation)	Presently important, however the added chemical is of environmental concern	Low	Adopted in this paper
Gravity settling	Hardly sinks after 3 h	-	Not applicable
Siphon type osmosis membrane	0.014% to 0.030% 1.7 min/kg-MA * (0.014%)	Membrane cost is high	Future possibilities

* Microalgae.

The environmental impact values were converted to CO₂ eq using the global warming potential in 100 years' time horizon (GWP 100) with SimaPro® v8.0.4. The SimaPro® is the name of LCA software developed by PRé Sustainability company located in the Netherlands.

Relevant data were collected from previous studies. Wastewater was introduced from a sewage facility as a nutrient for the microalgae. The production of microalgae was calculated based on the results obtained from the pilot plant of microalgae biofuel production in Minamisoma [4]. Subsequently, sufficient exhausted CO₂ and waste heat were assumed for algae fuel production.

The feasibility of wastewater treatment was integrated with microalgae growth culture [4]. In that experiment, the algal system successfully treated wastewater collected from the Kokaigawa Tobu WWTP in Ibaraki prefecture in December 2018. Before the treatment, the water exhibited a 5-day biochemical oxygen demand (BOD₅) of 52 mg/L, suspended solids (SS) of 140 mg/L, total nitrogen (TN) of 23 mg/L, and total phosphorus (TP) of 4.5 mg/L. Subsequently, after being treated by the algal system, the water exhibited a BOD₅ of 17 mg/L, SS of 18 mg/L, TN of 7.3 mg/L, and TP of 0.19 mg/L. Meanwhile, the water treated by the WWTP exhibited a BOD₅ of 11 mg/L, SS of 15 mg/L, TN of 9.1 mg/L, and TP of 1.4 mg/L. Both systems cleared the standard limit, which was a BOD₅ of less than 40 mg/L and an SS of less than 40 mg/L.

Moreover, the required amounts measured from the conventionally used artificial medium [7] were TN of 14.0 mg/L and TP of 1.2 mg/L. Compared with before the treatment, the BOD₅ was 52 mg/L, SS 140 mg/L, TN 23 mg/L, and TP 4.5 mg/L, and the TN and TP were sufficient for the inflow wastewater.

The algal scenario included emissions from wastewater treatment as a nutrient for the microalgae, CO₂ absorption at the ORP, and CO₂ emission in the HTL. Furthermore, the algal scenario included the use of CO₂ containing exhaust gas in the ORP and the use of discharged heat in the HTL process from power plants.

The water depth of the ORP for producing microalgae was 0.2 m. Furthermore, experiments in the Minamisoma plant revealed that it could grow even at a depth of 0.8 m. A difference in distance exists between the OD method (2.5 m) and algae ORP (0.8 m). However, the processing capacity of a WWTP using the OD method is generally half that of Tochigi prefecture. Some WWTPs using the OD method used only 3% of its capacity. WWTPs using the OD method are in a rural area. Therefore, if the system capacity is reduced to one third, and assuming that both HRTs are the same, the algal system can be introduced by reducing the depth of the OD from 2.5 to 0.8 m.

From the experimental analysis, it was discovered that the primary overflow water at a standard activated sludge method can be used for algal cultivation. However, in this scenario, the nonprimary treated water at the OD method was set instead of the primary overflow water. Consequently, the effect of algae growth may not be the same throughout the process.

2.2.1. Datasets for Simulation

To calculate the CO₂ emission and energy profit ratio (EPR) in the algal scenario, the following data were used in the simulation process. The data were based on the experiment at Minamisoma, from the input of 50 m³-wastewater (m³-w.w.) per day produced by 5.66 kg biocrude-wet. First, the calculation was done based on 50 m³-w.w./day and then divided the result by 50 to adapt to the 1 m³-w.w. of the algal scenario.

More than one hundred parameters had to be calculated in the algal scenario. For convenience, the parameters were categorized into independent, dependent, and fixed. The independent parameters imply that the parameters can be adjusted based on the situation. The dependent parameters change dynamically based on the independent and fixed parameters. The fixed parameter is fixed by experience and literature data (Table A1). The independent and dependent parameters are explained below, and part of the dependent parameters used in the functions (Tables A2 and A3).

2.2.2. Use of Previous Research Results

The calculation of the algal scenario's process in this study is a continuation of the theoretical energy analysis of algae fuel production [5]. The theoretical data was revised to experimental data using new experimental datasets (Table 3). The expanded calculation was performed to estimate the environmental effect.

In the algal scenario, cultivation, filtration, and extraction were used similarly as in the previous study. However, for the centrifugation process, the centrifuge equipment was replaced with a flocculation tank to reduce the energy input.

Moreover, a purification process was introduced. In a previous study [5], the HHV of biocrude-wet was set as 34.2 MJ/kg-biocrude-wet. However, in [4], the HHV of biocrude-dry was 29.1 MJ/kg-biocrude-dry for the microalgae purification in Minamisoma. Biocrude-wet was set as the final product for the flexibility of use for purifications that depend on the use purpose. Furthermore, the price is determined in the biocrude-wet condition, with consideration of moisture content.

Table 3. Analytical process for each stage of microalgae production from the pilot plant located at Minamisoma.

Process	Cultivation	Centrifugation	Filtration	Extraction	Purification
Previous study [5]	Empirical	Empirical	Experimental	Theoretical	-
This research	Empirical	Theoretical	Experimental	Experimental	Experimental

2.2.3. Design Parameter of Raceway Pond

The basic parameters were set to calculate the CO₂ emissions and EPR (Table 4). As mentioned previously, the independent parameters (Table A1) based on the situation (Figure 2) at Minamisoma, and the dependent parameters (Tables A2 and A3) were read from the independent parameters automatically. For example, the pump capacity (83 L/min) was calculated by the daily handling volume (50 m³) divided by the working hours C (pump)(10 h/d), and divided by 60 min, in which the daily handling volume (50 m³) was obtained from the pond volume (200 m³) divided by the HRT (4 d). All calculations were based on the handling of 50 m³-w.w. per day.

Figure 2. Raceway pond (1000 m² scale).

Table 4. Parameters set in the open raceway pond per 50 m³/day.

Parameter	Input	Unit
Independent		
Pond volume	200	m ³
Pond length	50	m
Pond width	20	m
Pond water depth	0.2	m
HRT	4	day
Dependent		
Pond area	1000	m ²
Pump capacity	83	L/min

2.2.4. Cultivation

In this section, the details of the parameters set and the mass balance for cultivation (ORP) in the algal scenario are provided (Table 5 and Figure 3). The wastewater was used as the liquid medium and nutrient, based on [4], which refers to the possibility of wastewater as a water and nutrient supplier for the algae. As mentioned above, the additional CO₂ (concentration 15%) was recycled from exhausted CO₂ by a power plant or garbage incinerator. CO₂ eq emissions were not observed. However, 55% (0.325 kg/m³-w.w.) of input CO₂ generated an output to the atmosphere without absorption. Therefore, a direct emission in the scenario was calculated.

Table 5. Parameters set at cultivation sub-unit per 50 m³/day.

Parameter	Input	Unit
Independent		
Water (Waste: 1 or Fresh: 0)	1	-
WWTP for algae	1	item
Pump	2	item
Pipe A	20	M
Dependent		
Acetic acid (50% from HTL)	15	kg/day
Carbon dioxide	15	kg/day
Wastewater	50	m ³ /day
Electricity for paddle wheel (e4)	36	MJ/day
Electricity for pump (e1)	37	MJ/day
Paddlewheel (n4)	1	item

Algal scenario

Figure 3. Mass balance of microalgae oil production in cultivation sub-unit per 1 m³.

In the dependent parameters, the brackets are marked “(e4), (e1), and (n4).” These brackets implied that they were determined by the formulated function. For example, the electricity for the

paddlewheel value of 36 MJ/day was calculated from (e4), $\{2.75 \times 0.2 \text{ m (Depth)}\} \text{ kW} \times 18 \text{ h/day} \times 3.6 \text{ MJ/kWh} = 36 \text{ MJ/day}$. Subsequently, electricity was added for a pump (Figure 4), and the total electricity was 72.7 MJ/day. The electricity of 72.7 MJ/day was calculated based on a one-day handling volume of 50 m^3 in Minamisoma. The calculation standard of the algal scenario is 1 m^3 . To divide by 50 m^3 for adopting 1 m^3 , the electricity consumption was observed 1.45 MJ/m^3 .

As mentioned in the Methodology, the exhausting nutrient from the HTL process was set in algal cultivation, because the exhausted nutrient could be used as a substitution of acetic acid [4]. Half of the acetic acid was replaced in this scenario. The total mass of acetic acid input was set at 0.3 g/L . Other masses of input, output, and waste were the same of those of a previous study [5]. The microalgae proceeded to the sedimentation process after cultivation.

Figure 4. Pumps for harvest in cultivation sub-unit at Minamisoma ($0.55 \text{ kW} \times 2 \text{ units}$).

2.2.5. Sedimentation

The flocculant sub-unit is a sedimentation process, described in the next sections of the cultivation system (Figure 1). In this unit, input is first received from the cultivation sub-unit and is concentrated in the flocculant tank. Subsequently, the output is passed on to the filtration sub-unit (Figure 5).

Figure 5. Mass balance of microalgae oil production in flocculant sub-unit per 1 m^3 .

The centrifuge equipment (Figure 6) was replaced with a flocculation tank to reduce the energy input. A polymer coagulant ($3 \text{ g/m}^3\text{-w.w.}$) for harvest (Sedimentation A) and a polymer coagulant ($0.5 \text{ g/m}^3\text{-w.w.}$) with Polytec solution ($0.25 \text{ kg/m}^3\text{-w.w.}$) can be used for treating the waste (Sedimentation B); additionally, electricity ($0.04 \text{ kWh/m}^3\text{-w.w.}$) instead of centrifuge ($1 \text{ kWh/m}^3\text{-w.w.}$) can be used in the same microalgae recovery rate [4] (Table 6). The surplus material was known as “waste” instead of the term “sludge” used in [5], but their contents were the same.

Figure 6. Centrifuge equipment at Minamisoma (15 kW, 8000 L/h).

Table 6. Parameters set at flocculant sub-unit per 50 m³/day.

Parameter	Input	Unit
Independent		
SS of after concentration	1.156	%
Biomass MA * passing rate	100	%
Rate of waste and output	97.12	%
Flocculant tank capacity	3	m ³ /h
Flocculation tank	1	item
Pump	1	item
Dependent		
Input mass of biomass MA *	50,031	kg/day
SS of before flocculant	0.034	%
Electricity for flocculant (e2)	8	MJ/day
Polytec solution	12.5	kg/day
Polymer coagulant	0.175	kg/day

* Microalgae.

2.2.6. Filtration

In the drum filtration sub-unit, algae were concentrated by the pressure of the drum filter (Figure 7). In this unit, the input from the flocculant sub-unit was passed to the HTL sub-unit (Figure 8). The biomass microalgae (MA) passing rate was set at 98.02%, while it was set as 100% in the flocculant sub-unit because some biomass remained in the filter and were beyond the system boundary (Table 7).

Figure 7. Drum filter equipment at Minamisoma (1.42 kW, 520 kg/h).

Algal scenario **Figure 8.** Mass balance of microalgae oil production in drum filtration sub-unit per 1 m³.**Table 7.** Parameters set at filtration sub-unit per 50 m³/day.

Parameter	Input	Unit
Independent		
SS of after concentration	20	%
Biomass MA passing rate	98.02	%
Rate of emission and output	95.09	%
Drum filtration	1	unit
Drum filtration-capacity	300	kg/h
Biomass conveyor	1	m
Dependent		
Input mass of biomass MA	1441	kg/day
SS of before flocculant	1.156	%
Drum filtration-usage rate	27	%
Electricity for drum (e3)	25.8	MJ/day

2.2.7. Extraction (HTL)

In this HTL sub-unit (Figure 9), the input from the drum filtration sub-unit produces the biocrude-wet (Figure 10). The number of HTL units was set as three by the daily handling mass of microalgae and the working hours (Table 8). In the HTL process, the waste heat energy was used, which required more than 350 °C (8.59 MJ/m³-w.w.). The CO₂ emissions of 0.053 kg implied a direct CO₂ emission in the HTL process, which differed from the calculated total CO₂ eq. The direct CO₂ emission was calculated from biomass stoichiometry. After the HTL sub-unit, 113 g of biocrude-wet was produced. The biocrude-wet contained H₂O. After drying the H₂O by purification, 74.7 g of biocrude-dry (HHV: 2.17 MJ/kg) was produced. However, this purification method and output ratio were different by purpose to use the biocrude. For example, in the desalter process before the distillation process in the petroleum refining industry, water is added to crude oil for desalting. Hence, the calculation was set based on biocrude-wet, which is before the purification.

Figure 9. HTL equipment at Minamisoma (10.2 kW, 21.6 kg/day).

Figure 10. Mass balance of microalgae oil production in HTL sub-unit per 1 m³.

Table 8. Parameters set at extraction (HTL) sub-unit per 50 m³/day.

Parameter	Input	Unit
Independent		
HTL system (n5)	3	unit
HTL conversion ratio	8.0	%
Pipe B	15	m
Pump	1	item
Dependent		
Input mass of biomass MA	70.81	kg/day
SS of before flocculant	20	%
Electricity for HTL heater	(430)	MJ/day

3. Results and Discussion

Using the parameters above, the environmental impact and EPR of the two scenarios were estimated.

3.1. Environmental Impact of Existing Scenario

3.1.1. Wastewater Treatment

To estimate the environmental impact of the WWTP, the technical note of the National Institute for Land and Infrastructure Management [10] was used. In the case study from the technical note, 560,164 m³ of wastewater was treated in the WWTP using the OD method in 2014. The environmental impact during wastewater treatment operation was 0.884 kg-CO₂ eq/m³-w.w. and the energy demand per processing volume was 17.5 MJ/m³-w.w., in which electricity, heavy oil, caustic soda, polymer flocculants, tap water, LPG, and solid chlorine were included. However, in this algal scenario's system boundary, the disinfection process was not included (Figure 1). Similarly, the emission from solid chlorine (0.00038 kg-CO₂ eq/m³-w.w.) for disinfection was subtracted from the entire emission. The effect was small compared with the entire emission; therefore, it did not affect the result.

3.1.2. Sludge Cake Disposal

The environmental impact of sludge cake treatment was calculated by the sludge volume, sludge cake output ratio, and CO₂ eq emissions by a high-temperature incineration of the sludge cake. To calculate the sludge cake output ratio, the amount of annual wastewater treatment in each OD method plant was obtained for fiscal year 2011 (Figure 11). 20 WWTPs in Tochigi prefecture were selected, which used the OD method and whose processing start date was after 1989 (30 years ago) [11]. The old treatment plants (before 1988) were excluded because they involved singular values. Subsequently, the input wastewater was represented on the x-axis and the output of the dehydrated

sludge discharge on the y-axis. Hence, an approximate expression was obtained. From that expression, the dehydrated sludge of 1 m³ of wastewater was estimated to be 690 g.

Figure 11. Annual average input of wastewater and related output of sludge cake.

The CO₂ eq emissions per dehydrated sludge (DS) treatment was observed at 2.697 t-CO₂ eq/t-DS [12]. In this case, the DS differs from the algae's dry-solid, which refers to a dehydrated sludge that is not completely dry. The data was from a case study of the Wakagawa sewage treatment plant in Wakayama prefecture, Japan, in FY 2010. This DS was treated by high-temperature incineration under an annual sludge treatment of 5347 t/y, with 81.8% water content.

As a result of the environmental impact from 1 m³-w.w., 0.69 kg-DS × 2.697 kg-CO₂ eq/kg-DS = 1.86 kg-CO₂ eq/m³-w.w. was emitted.

3.1.3. Import of Crude Oil

The environmental impact of crude oil was based on the total amount of emissions excluding self-combustion and domestic transport, i.e., 5.63 g-C eq/Mcal (0.001345 kg-C eq/MJ) [13]. Because the unit of calorific value of crude oil is 38.28 MJ/L [9], the environmental impact of per liter import for crude oil was calculated from 0.001345 kg-C eq/MJ × 38.28 MJ/L = 0.0515 kg-C eq/L. The specific gravity of the crude oil used was 0.854 kg-crude-oil/L [14]. After changing the unit, the environmental impact was 0.0603 kg-C eq/kg-crude-oil.

0.0603 kg-C eq/kg-crude-oil was multiplied by the coefficient of 3.664 for the conversion of carbon to carbon dioxide and obtained 0.221 kg-CO₂ eq/kg-crude-oil, which is the amount of CO₂ eq emissions for import to Japan per kilogram of crude oil. Therefore, 48.4 g of crude oil resulted in 0.0107 kg-CO₂ eq of emissions.

3.1.4. Total CO₂ Emission

The results of the environmental impact from the existing scenario were revealed that the total CO₂ emission in the existing scenario was 2.76 kg-CO₂ eq/m³-w.w., which included the emission from the 48.4 g of imported crude oil (Figure 12).

Figure 12. Environmental impact of GWP 100 from operating emissions of the two scenarios.

3.1.5. EPR

In the existing scenario, the energy requirement was set from the case study. Subsequently, the yearly operating energy from electricity in the OD method was 7,691,609 MJ/560,164 m³-w.w. (13.73 MJ/m³-w.w.) [10]. In this case, the energy from electricity included the electricity for screen and disinfection. However, the system boundary of the existing scenario did not include the electricity for screen and disinfection. Therefore, the adjustment was required. One example showed that 74% of the electricity at the WWTP using the OD method was used for water treatment, sludge concentration, and sludge dehydrating [15]. Based on that example, at least an energy of 13.73 MJ/m³-w.w. × 74% = 10.16 MJ/m³-w.w. was required in the existing scenario (Table 9).

Table 9. Operating energy balance of each scenario.

	Energy MJ/m ³ -w.w.
Existing scenario	
Input	10.16
Output (Crude oil)	2.17
(EPR)	0.21
Algal scenario	
Input	5.32
Output (Algal biocrude)	2.17
EPR	0.41

Although it was not called the EPR, if the calorific value of crude oil (2.17 MJ/m³-w.w.) was divided by the input energy (10.16 MJ/m³-w.w.), the ratio was 0.21.

3.2. Environmental Impact of Algal Scenario

3.2.1. Algae Fuel Production

For algae fuel production, the usage of equipment with energy consumption and the input of materials were calculated and then converted to 1 kilogram of biocrude-wet production. The conversion results were analyzed with SimaPro[®] version 8.0.4. using methods was “CML-IA baseline” version 3.02 (Table A4). The total environmental impact of the operating energy was 19.2 kg-CO₂ eq/kg-biocrude-wet (Table 10). Subsequently, 1 m³ of wastewater produced 113 g of biocrude-wet, which resulted in 2.17 kg-CO₂ eq/m³-w.w. of emissions.

Table 10. Emission from algal fuel production.

Process		Operating Emission	
		kg-CO ₂ eq/kg-Biocrude-Wet	kg-CO ₂ eq/m ³ -w.w.
ORP (Cultivation)	System	6.4	0.72
	Exhaust gas	5.5	0.63
Flocculant		6.6	0.75
Drum filtration		0.7	0.08
HTL		(11.8)	(1.34)
Total		19.2	2.17

Most of the emission was from electricity. The cultivation was the highest emitted process in the algal scenario. The process used not only the electricity from the paddlewheels and pump, but also acetic acid and carbon dioxide as direct materials.

From an annual report [4], the microalgae CO₂ use rate from exhaust gas was assumed to be 45% (Figure 13). The mass balance of the CO₂ absorption per 1 m³-w.w. was calculated from the experimental dataset, which compared the difference between the use of exhaust gas and without it. Consequently, the suggested algae CO₂ absorption ratio of exhaust gas to the atmosphere was 8:2. When the algae absorbed 0.639 kg-CO₂/m³-w.w., 80% (0.511 kg-CO₂/m³-w.w.) was from the exhaust gas. Meanwhile, 20% (0.128 kg-CO₂/m³-w.w.) was from the atmosphere. From the side of the input exhaust gas, 45% (0.511 kg-CO₂/m³-w.w.) was absorbed by the algae and 55% (0.625 kg-CO₂/m³-w.w.) was emitted to the atmosphere. CO₂ contains was set as 15% of exhaust gas in this calculation.

Algal scenario

Figure 13. Mass balance of CO₂ in ORP (kg-CO₂/m³-w.w.) within 4 d of HRT.

The CO₂ used in the ORP can be obtained without purchasing by using exhaust gas from power plants. Furthermore, CO₂ can be used without any environmental degradation. Meanwhile, 55%

(0.625 kg-CO₂/m³-w.w.) of the input amount of exhaust CO₂ was included as emissions during the ORP process (1.34 kg-CO₂ eq/m³-w.w.). Consequently, if the emission from the exhausted CO₂ was discounted, the emissions from the cultivation pond could be 0.72 kg-CO₂ eq/m³-w.w. (Table 9). The total emissions could also be 1.54 kg-CO₂ eq/m³-w.w. from 2.17 kg-CO₂ eq/m³-w.w.

In the use of waste heat in the HTL, the heat energy of 8.6 MJ/m³-w.w. was calculated from waste heat without applying an environmental load.

3.2.2. CO₂ Absorption at Pond

Microalgae can fix CO₂ from the air, or exhaust gas could be introduced for photosynthesis in the ORP system. Therefore, microalgae might be set as a CO₂ absorption source. For example, it is assumed to correspond to the "revegetation" part in the Kyoto Protocol [16]. Hence, the CO₂ absorption was set by the microalgae biomass stoichiometry [5].

The stoichiometry of function (1) shows the microalgae CO₂ absorption from the air, where 1.88 g of CO₂ was absorbed in 1 g of microalgae (Table A5 in Appendix B). In the case of 1 m³ of wastewater for growing the algae, with the harvest concentration of 0.034% (0.34 g/L-DCW), the absorbed CO₂ was estimated to be 0.34 kg-DCW/m³ × 1.88 kg-CO₂/kg-DCW = 0.64 kg-CO₂/m³-w.w. The microalgae can be absorbed CO₂, HNO₃, H₂SO₄, H₃PO₄, and H₂O. From those materials, only CO₂ was calculated in this study. Consequently, the CO₂ equivalent absorption at the pond was 0.64 kg-CO₂ eq/m³-w.w.

3.2.3. HTL Process

In the HTL process, it is presumed that the microalgae biomass stoichiometry (2) is according to the results of experimental data [17] (Table A6 in Appendix B). In the conversion process, 1 m³ of wastewater was transformed to 1.42 kg with 20% algae. In such a condition, the HTL process emission of CO₂ was (1.42 kg × 20%)/m³-w.w. × 0.186 kg-CO₂ = 0.053 kg-CO₂/m³-w.w. Additionally, NH₃ was emitted from this process; however, NH₃ is carbon-free. Subsequently, the total CO₂ equivalent of this process was 0.053 kg-CO₂ eq/m³-w.w.

3.2.4. Total CO₂ Emission

In the algal scenario, an emission of 1.59 kg-CO₂ eq/m³-w.w. occurred when treating 1 m³ of wastewater to produce 113 g of biocrude-wet. Compared with the existing scenarios, the total CO₂ eq emissions (2.76 kg-CO₂ eq/m³-w.w.) in the algal scenario could reduce the environmental impact significantly.

The primary contributor to increase the emission in algae fuel production was the narrow scale of the production system. The base scale of the WWTP in the existing scenario was 560,164 m³/y, but that of the algal scenario was 16,500 m³/y (50 m³/d), i.e., only 3%. If the algal scenario in the algae fuel production was scaled up by 30x (1500 m³/d), for comparing in the same volume. The emission of 2.17 kg-CO₂ eq/m³-w.w. from the algae fuel production was estimated as 2.03 kg-CO₂ eq/m³-w.w. However, land constraints must be considered. The CO₂ emission of 0.625 kg from exhaust gas was included in the CO₂ emission of the algae fuel production. In some cases, this number can be subtracted.

Some aspects are included, while others are excluded in the calculation of GWP 100 by each process (Table 11). In general, CH₄ and N₂O are directly emitted from the treatment of wastewater [18]. However, the emissions were almost the same because the treated amount of wastewater was the same as that of a similar system. Hence, both scenarios did not include the emission from them.

Table 11. Included and excluded matters in the calculation of GWP 100.

Process	Type	Include	Exclude
Wastewater treatment	CO ₂ eq	Direct energy and material	Illumination, air conditioning, solid chlorine, CH ₄ , N ₂ O
Sludge cake disposal	CO ₂ eq	Self-combustion	Transport
Import of crude oil	CO ₂ eq	Overseas transport	Domestic transport
Algal fuel production	CO ₂ eq	Direct energy and material	Illumination, air conditioning, solid chlorine, CH ₄ , N ₂ O
Absorption at pond	CO ₂	Direct CO ₂ absorption by photosynthesis	Exhalation by breathing
HTL process *	CO ₂	Direct CO ₂ emission	Direct energy

* HTL process “energy” was explained at algal fuel production.

3.2.5. EPR

In the algal scenario, the outline of the in-out energy was verified, which did not include the energy for lighting, air conditioning, and electrical devices. Furthermore, the electricity for the screen and disinfection process was not included. Consequently, the treatment of 50 m³ of wastewater producing 5.66 kg of biocrude-wet required 106.5 MJ of electricity (Table 12). Therefore, the treatment of 1 m³ of wastewater required 2.13 MJ of electricity.

Table 12. Operating electricity from algal fuel production.

Process	Electricity MJ/50 m ³ -w.w.	Electricity MJ/m ³ -w.w.	Energy MJ/m ³ -w.w.
ORP (Cultivation)	72.7	1.45	3.63
Flocculant	8.0	0.16	0.40
Drum filtration	25.8	0.52	1.29
HTL	(429.6)	(8.59)	(21.5)
Total	106.5	2.13	5.32

To calculate the in-out energy, the power generation efficiency was set as 40%. Therefore, the required energy was calculated from electricity, i.e., 5.32 MJ/m³-w.w. The output from 1 m³ of wastewater was 113 g of biocrude-wet, which is the same energy level as that of 74.7 g biocrude-dry. In this conversion process, the HHV was set as 29.1 MJ/kg-biocrude-dry. Consequently, that can produce 74.7 g × 29.1 MJ/kg = 2.17 MJ/m³-w.w. Hence, the EPR was 0.41 (Table 8), which implied that 40% of the input energy for the wastewater treatment could be recycled. Compare with the existing scenarios EPR 0.21, the algal scenario could reduce the operating energy requirements.

4. Conclusions

This study concludes that the microalgae’s realistic scenario using the discharged heat could be effective in treating general wastewater and emitted CO₂, in terms of EPR and CO₂ emissions. The EPR was 0.41 in the algal scenario as compared with the existing scenario’s 0.21. In addition, the environmental impact could be reduced from 2.76 to 1.59 kg-CO₂ eq/m³-w.w. This is combined with 1 m³ of wastewater treatment, waste heat use from power plants, and CO₂ absorption in algae production. The waste heat use in the HTL process was 21.5 MJ/m³-w.w. A comprehensive and realistic whole scenario from the cultivation process to biocrude purification was proposed for the first time in terms of environmental impact and energy balance as presented by this study.

Moreover, in the HTL process, experimental data from the Minamisoma plant showed that 113 g of biocrude-wet was produced from 1.42 kg of 20% microalgae (280 g dry weight). Meanwhile, based on stoichiometry, 213 g of biocrude-wet could be produced from 280 g of dry weight of microalgae. This indicated that the experimental value was only half the theoretical value. If the HTL process yielded

the theoretical value, then the operating EPR would increase from 0.4 to 0.8 including wastewater treatment. Hence, the EPR could reach 1.0 by improving the efficiency of cultivation, sedimentation, filtration, HTL extraction of microalgae, and scale-up of ORP from 50 m³/day, as well as changing the production area to a warm place where algae could grow easily.

In this study, the algal scenario exhibited some limitations to scale up the pilot plant to the commercialization scale. In general, a system with low energy consumption and low GHG emissions tend to have lower costs. Therefore, it can be assumed that the algal scenario is more economical than the existing scenario. Further studies are required not only the environment but also the economical optimization together, to push up the commercialization stage of the algae scenario.

Author Contributions: This work was developed and written by R.I.; A.S.P. evaluated the technical calculation. It was conceived with R.N., S.I., T.A. and M.M.W., who provided major editorial contributions and guidance. All authors contributed to the writing of the paper. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

The data set to estimate the environmental impact in the algal scenario, which collected from the Minamisoma pilot plant.

Table A1. Independent parameters for LCA calculation.

Parameter	Input	Unit
0. Construction of raceway pond		
Working days	330	day/year
Pipe A	721	JPY/m
Pipe A depreciation	3	year
Pipe B (HTL)	721	JPY/m
Pipe B depreciation	2	year
Pump depreciation	3	year
Paddlewheel price	50000	JPY/item
Paddlewheel depreciation	5	year
Biomass conveyor price	10000	JPY
Biomass conveyor depreciation	2	year
Working hours, A (System)	24	h/day
Working hours, B (Equipment)	18	h/day
Working hours, C (Pump)	10	h/day
1. ORP sub-unit		
Average operating energy of WWTP	0.065	L eq-oil/m ³
Scale merit of concrete (Based on 0.1 ha)	0.7	-
Concrete (ORP) depreciation	30	year
WWTP average construction cost	730000	JPY/m ³
WWTP depreciation	20	year
Transformation to industrial area-depreciation	100	year
2. Flocculant sub-unit		
Flocculant tank depreciation	20	year
Polytec solution price	34.1	JPY/kg
Polymer coagulant price	34.1	JPY/kg
3. Drum filtration sub-unit		
Drum filtration depreciation	10	year
Drum filtration scale merit	1	-
Biomass conveyor scale merit	1	-

Table A1. Cont.

Parameter	Input	Unit
4. HTL sub-unit		
HTL heater in Minamisoma (2.2, 2, 2.5 x 2, 0.5 x 2)	10.2	kW
Working time of HTL heater in Minamisoma	3.9	h/day
HTL equipment price	4 M	JPY/unit
HTL capacity	21.6	kg/day/unit
HTL depreciation	10	year
5. General information		
CO ₂ credit	1.845	JPY/kgCO ₂
Electricity	27	JPY/kWh
1kWh	3.6	MJ/kWh
Power generation efficiency	40	%
The CO ₂ emission factor of B and C heavy oils	3	kgCO ₂ /L-heavy oil
1 Barrel (Throughput oil)	140	kg
1 Btu (British thermal unit)	1054	MJ
Labor costs	500 M	JPY/year
Acetic acid price	55	JPY/kg
Acetic acid using ratio	0.3	g/L
Freshwater	213	JPY/m ³
Volume of concrete for pond	415.5	m ³ /1000 m ²
Exchange rate (Dec 17, 2018)	114	JPY/USD
Exchange rate (Dec 17, 2018)	126	JPY/EUR

Table A2. Function list of “electricity” for dependent parameters. (x is derived from Table A1).

No.	Parameters $f(x)$	Unit	Equation $f(x) = ax + b$	x	unit	a	b	R ²
e1	Pump	kW	$f(x) = 0.0029x + 0.2725$	Capacity	L/min	0.0029	0.2725	1.00
e2	Flocculant machine	kW	$f(x) = 0.5972x + 0.5611$	Capacity	m ³ /h	0.5972	0.5611	0.89
e3	Drum filter machine	kW	$f(x) = 0.0202x + 1.4161$	Capacity	m ³ /h	0.0202	1.4161	0.94
e4	Paddlewheel	kW	$f(x) = 2.75x$	Depth	m	2.75	0	1.00

Table A3. Function list of “number of items and workers” for dependent parameters. (x is derived from Table A1)

No.	Parameters $f(x)$	Original	Equation $f(x) = ax,$ $f(x) = a \times \text{Ln}(x) + b$	x	unit	a	b
n1	Pump	2+1+1	Capacity change		item		
n2	Flocculant machine	1	Capacity change		item		
n3	Drum filter machine	1	Capacity change		unit		
n4	Paddlewheel	1	$f(x) = \text{Roundup}(x/1000)$	Pond area	m ²	1000	-
n5	HTL unit (20mL/min)	1	$f(x) = \text{Roundup}(x/21.6)$	Biomass MA (20%)	kg/day	21.6	-
w1	Worker	2	$f(x) = \text{Round}(0.7135 \times \text{Ln}(x/10000) + 3.5714, 0)$	Pond area	m ²	0.7135	3.5714

Table A4. Input and output data used in the simulation process using SimaPro® (Per 1 kg-biocrude-wet from 8.8 m³-w.w.).

1 Cultivation	Input Item	Unit	Quantity		kg-CO ₂ eq
			Operate	Total	Operate
Output	Biomass MA (0.034%)	kg	8834.70	8834.70	
Direct material	Acetic acid	kg	2.65	2.65	4.35
	Carbon dioxide, in the exhaust gas	kg	4.52	4.52	
Direct energy	Electricity (Paddle)	MJ	6.30	6.30	0.98
	Electricity (Pump)	MJ	6.54	6.54	1.02
Building and Equipment	Concrete	m ³		0.00185	
	WWTP	unit		0.0000268	
	Paddlewheel	item		0.000107	
	Pump	item		0.000357	
	Pipe	m		0.00357	
Land occupation	Transformation to industrial area	m ²		0.00535	
Emission	Carbon dioxide (Emission: 55%)	kg	5.52	5.52	5.52
2 Sedimentation			Total CO ₂		11.9
Output	Biomass MA (1.156%)	kg	254.46	254.46	
Input	Biomass MA (0.034%)	kg	8834.70	8834.70	
Direct material	Polytec solution	kg	2.21	2.21	6.30
	Polymer coagulant	kg	0.0309	0.0309	0.08
Direct energy	Electricity (Flocculation)	MJ	1.41	1.41	0.22
Equipment	Flocculation tank	unit		0.0000268	
	Pump	item		0.000178	
Sludge		kg	8580.24	8580.24	
3 Filtration			Total CO ₂		6.6
Output	Biomass MA (20%)	kg	12.50	12.50	
Input	Biomass MA (1.156%)	kg	254.46	254.46	
Direct energy	Electricity (Drum filtration)	MJ	4.55	4.55	0.71
Equipment	Drum filtration	item		0.0000143	
	Biomass conveyor	m		0.000268	
Sludge		kg	241.95	241.95	
4 Extraction (HTL)			Total CO ₂		0.7
Output	Biocrude-wet	kg	1	1	
Input	Biomass MA (20%)	kg	12.50	12.50	
Direct energy	Electricity (HTL)	MJ	(75.86)	(75.86)	(11.8)
Equipment	HTL equipment	item		0.000175	
	Pipe	m		0.00401	
	Pump	item		0.000178	
			Total CO ₂		0

Appendix B

The data set collected from the literature in the algal scenario.

Table A5. Microalgae biomass stoichiometry at ORP process [7].

	CO ₂ +	0.148HNO ₃ +	0.014H ₂ SO ₄ +	0.012H ₃ PO ₄ +	0.751H ₂ O →	CH _{1.715} O _{0.427} N _{0.148} S _{0.014} P _{0.012} +	1.437O ₂
Mass (g)	22,507	4769	702	601	6919	12,000	23,515
Ratio	1.88	0.40	0.06	0.05	0.58	1.00	1.96

Table A6. Microalgae biomass stoichiometry at HTL process [17].

	1000 C _{3.81} H _{6.83} N _{0.57} O _{1.51}	→	754C _{4.58} H _{8.05} N _{0.42} O _{1.05} +	359CO ₂ +	253NH ₃ +	H ₂ O
Mass (g)	84,788		64,697	15,800	4310	18
Ratio	1.00		0.76	0.186	0.051	0.00021

References

1. Sasongko, N.A.; Noguchi, R.; Ito, J.; Demura, M.; Ichikawa, S.; Nakajima, M.; Watanabe, M.M. Engineering Study of a Pilot Scale Process Plant for Microalgae-Oil Production Utilizing Municipal Wastewater and Flue Gases: Fukushima Pilot Plant. *Energies* **2018**, *11*, 1693. [CrossRef]
2. Demura, M.; Yoshida, M.; Yokoyama, A.; Ito, J.; Kobayashi, H.; Kayano, S.; Tamagawa, Y.; Watanobe, M.; Date, N.; Osaka, M.; et al. Biomass productivity of native algal communities in Minamisoma city, Fukushima Prefecture, Japan. *Algal Res.* **2018**, *29*, 30. [CrossRef]
3. Iasimone, F.; de Felice, V.; Panico, A.; Pirozzi, F. Experimental study for the reduction of CO₂ emissions in wastewater treatment plant using microalgal cultivation. *J. CO₂ Util.* **2017**, *22*, 1–8. [CrossRef]
4. Algae Industry Incubation Consortium Japan. *Annual Report of the Microalgae Pilot Project for Biofuel Production*; ABES University of Tsukuba: Tsukuba, Japan, 2019; pp. 29, 35, 44, 45, 58, 110. (In Japanese)
5. Wibawa, D.S.; Nasution, M.A.; Noguchi, R.; Ahamed, T.; Demura, M.; Watanabe, M.M. Microalgae Oil Production: A Downstream Approach to Energy Requirements for the Minamisoma Pilot Plant. *Energies* **2018**, *11*, 521. [CrossRef]
6. Kodo, S. *Sewer Book*, 1st ed.; The Nikkan Kogyo Shimbun, LTD.: Tokyo, Japan, 2012; pp. 88–89. (In Japanese)
7. Algae Industry Incubation Consortium Japan. *Annual Report of the Microalgae Pilot Project for Biofuel Production*; ABES University of Tsukuba: Tsukuba, Japan, 2018; pp. 18, 19, 30. (In Japanese)
8. Gohara, T.; Jain, A.; Matumoto, S.; Aramaki, T.; Ito, J.; Noguchi, R.; Watanabe, M.M.; Nakajima, M.; Ichikawa, S. Continuous Production of Biocrude by Hydrothermal Liquefaction of Native Microalgae. In Proceedings of the 14th Biomass Science Conference, Higashihiroshima, Japan, 16–18 January 2019; Biomass Division of the Japan Institute of Energy: Tokyo, Japan, 2019; pp. 91–92. (In Japanese).
9. Table of Standard Calorific Value and Carbon Emission Coefficient. Available online: https://www.enecho.meti.go.jp/statistics/total_energy/pdf/stte_016.pdf (accessed on 28 September 2019).
10. National Institute for Land and Infrastructure Management. Guideline for Life-Cycle Assessment of Sewerage Systems. In *Technical Note of National Institute for Land and Infrastructure Management*; NILIM Ministry of Land, Infrastructure, Transport, and Tourism: Tsukuba, Japan, 2010; pp. 97, 105. (In Japanese)
11. Tochigi Prefecture Prefectural land maintenance business meeting. *Public Sewage of Tochigi*; Tochigi prefecture: Utsunomiya, Japan, 2013; pp. 3–4. (In Japanese)
12. Nakao, A.; Yamamoto, Y.; Yoshida, N. Evaluating Integration and Collaboration of Environmental Infrastructures and Choice of Low Carbon Technology in Declining Population Society. *J. JSCE. Ser. G (Environ. Res.)* **2015**, *71*, 480. (In Japanese)
13. Yoon, Y.S.; Yamada, T. Life Cycle Inventory Analysis of Fossil Energies in Japan. *Energy Econ.* **2000**, *25*, 22. (In Japanese)
14. How to Set the Fuel Constant. Available online: http://www.jari.or.jp/Portals/0/jhfc/data/report/2005/pdf/result_ref_1.pdf (accessed on 28 September 2019).
15. Ishida, T.; Ochi, S.; Otsuki, S.; Tamura, T. Joint Research on Energy-Saving Technology of Oxidation Ditch Method. In *Annual Report of JIWET 2016*; Eto, T., Ed.; Japan Institute of Wastewater Engineering and Technology: Tokyo, Japan, 2017; p. 176. (In Japanese)
16. Definitions, Modalities, Rules and Guidelines Relating to Land Use, Land-Use Change and Forestry Activities Under the Kyoto Protocol. p. 58. Available online: <https://undocs.org/FCCC/CP/2001/13/Add.1> (accessed on 28 September 2019).
17. Ankit, J. Solvent Extraction and Continuous Hydrothermal Liquefaction of Native Microalgae for Biofuel Production. Ph.D. Thesis, University of Tsukuba, Tsukuba, Japan, July 2018; p. 46.
18. Sano, I.; Masuda, S.; Li, Y.; Nishimura, O. Greenhouse Gases Emission Factors and it's Reduction Measures in Sewage Treatment Plant. *J. JSCE Ser. G (Environ. Res.)* **2012**, *68*, 568–569. (In Japanese)