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Original Paper

Nutrition Characterization of Aqueous Phase Produced by the Hydrothermal Treatment of Microalgae

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To mitigate the energy crisis and the environmental damage from fossil fuels exploration to combustion, much attention has been focused on liquid biofuel production from algae to reduce CO₂ emission and dependence on petroleum. Among the existing algae-to-biofuels technologies, the hydrothermal treatment has been proposed as an attractive one since the produced bio-oil has lower oxygen content than pyrolysis, and the dewatering step of the feedstock is not necessary. However, much biomass residue will be produced associated with the oil extraction from algae, which should be discharged as wastes. Especially the aqueous fraction accounts for the major part of the products. In this study, a green microalgae obtained from TISTR (Thai Institute of Science and Technology Research), named No.8511, was processed under different hydrothermal conditions to investigate the properties of aqueous phase products in terms of the nutrient content. Hydrothermal treatment was conducted at different concentrations of algae (from 5 % to 30 %) and temperature ranges (from 210 °C to 290 °C). The major nutrition elements and trace metal elements in the aqueous phase were quantified and analyzed. The results showed that a considerable nutrition recovery in aqueous part is possible.

Key Words

Microalgae, Bio-oil, Aqueous co-products, Nutrition elements analysis

1. Introduction

Efforts towards exploring a sustainable and environment-friendly alternative energy feedstock to liquid transportation fuel started from 1970s due to the oil crisis. Researchers have been producing petroleum-like biofuels from renewable biomass for three generations. Algae, the third generation biofuel source, stands out from other carbon neutral plants because of its superior high growth rate and high-quality, which is rich in lipid content and lack of structural support like lignocellulose, so that a grinding process is avoidable. Moreover, these aquatic organism does not require arable land and even can be cultivated in wastewater.

Energetically, the hydrothermal treatment is an ideal route for this wet biomass in contrast to other thermal conversion, like pyrolysis and gasification¹⁾. It saves a large amount of latent heat of vaporization of water, which

corresponds to 28.25 MJkg⁻¹ of oil produced by pyrolysis from 80 %-water-content algae sample, using all the assumptions set in the paper of Sawayama²⁾. Also, it was reported that the bio-oil produced from the hydrothermal treatment is not miscible with water and has a lower oxygen content, thus having a higher heating value than pyrolysis-derived oils possibly due to the dehydration of oxygen heteroatom and decarboxylation³⁾.

However, much spent biomass have to be disposed as wastes after bio-oil extraction especially the aqueous part. According to a hydrothermal treatment of *S. platensis* in 20 % algae slurry content, 39.93 ± 1.49 % of bio-oil, 4.67 ± 0.17 % solid residues were produced⁴⁾. But the total aqueous phase recovered was approximately 420 % of the starting biomass, which indicates the aqueous part is the key co-product that affects the whole economic feasibility of bio-fuel production. Previous studies generally focused on characteristics of bio-oil, with the yield distribution of other products, including gas, aqueous fraction and solid residue^{5) 15)}. A few studies have investigated the aqueous phase from hydrothermally

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treated algae as cultivation medium, in terms of the limiting factors of algae growth such as the dilution rate ^{4) 6)}, the toxicity by too high concentration of $\text{NH}_4^+\text{-N}$ and/or organic compounds ⁷⁾. The purpose of this study is to investigate only aqueous fraction and find out potential of aqueous recovery in different conditions. The nutritious elements and their distribution under the variables of algae loading rate and different heating temperature were analyzed and quantified respectively.

2. Experimental

2.1 Feedstock

TISTR8511 (a green microalgae obtained from Thai Institute of Science and Technology Research) was selected as the feedstock in a dry powder. The lipid content was determined by Bligh and Dyer method. The ultimate analysis was done using an analyzer (Vario MICRO Cube, Elementar Inc.) for CHONS. The trace element was determined by the ICP emission spectroscopy (ICPS-8100 Shimadzu Inc.) and ICP-MS (Elan DRC-e) after the digestion by the Multiwave3000 PerkinElmer Inc. **Table 1** summarizes the characteristics of dry algae powder. Then the algae powder was mixed with distilled water to make a slurry with a series of solid content for the hydrothermal treatment.

2.2 Hydrothermal treatment

The hydrothermal experiment was carried out by a stainless batch autoclave (MMJ-500, Japan) with a 500 ml SiO_2 reactor tube filled with algae slurry. This study investigated the relatively lower algae loading rate: 5, 10, 15, 20, 25 and 30 g algae powder was mixed with distilled water to make a series of algae slurry ranging from 5 to 30 %. Then they were heated to a temperature of 250 °C and kept for 30 min. For the study on the effect of

treatment temperature, 10 g algae powder was mixed with 90g distilled water with 30 min reaction time. The target reaction temperatures were 210, 230, 250, 270 and 290 °C.

The temperature was selected by economic consideration and prior researches. According to some researches reviewed by Akhtar and Nor Aishah ⁸⁾, the bio-oil yield increases a lot from 200 °C to 250 °C, but no big difference from 250 °C to 300 °C. For example, in the study of Zhou *et al.* ⁹⁾, the bio-oil produced at 220 °C to 320 °C increased from 9.6 to 20.4 wt%, but the yield at 260 °C was around 18 %. Meanwhile, Zhou *et al.* and Yu *et al.* ¹⁰⁾ reported the water soluble fraction increased until 240 °C, 230 °C respectively, then decreased along with the temperature increase. To produce more liquid products including oil and water soluble fraction, the temperature series from 210 °C to 290 °C was selected. The algae loading rate of 10 % was chosen also for the same reason. Theoretically, a subcritical temperature around 250 °C is also favorable for liquefaction. The ion product of water reaches the maximum near 250 °C, which means the amount of ion products used as catalyst is three times of that at the ambient temperature. The dielectric constant is 27.1 F/m at 250 °C, which is considerable as organic solvents. So the temperature 250 °C was chosen for the investigation of algae loading rate.

Before heating up, argon was fed into the reactor to purge out air. Then the reactor was stirred at 200 rpm and heated at 4.7 °C /min. After the treatment was finished, the reactor was not opened until the autoclave was depressurized and cooled down under 60 °C. The gas was released through the vent. Two or three replicates were conducted for each experimental condition, and the average values are shown in the results.

2.3 Products separation and aqueous analysis

The procedure following the hydrothermal treatment is depicted in **Fig. 1**. 100 mL (including washing part) dichloromethane (DCM, Sigma-Aldrich, 99 % purity) was added to the products and was intensively mixed. Then the mixture was filtered by a 1.2 μm pore-size glass microfiber filter paper (GF/C, whatman) and the separation was accelerated by using a vacuum pump. The filtered algal residue was defined as solid residue and quantified after the drying process. After sufficient sedimentation, the left two-phase liquid was further separated to DCM phase (bottom part) and upper aqueous phase (water soluble phase) by an auto-pipette (Gilson Pipetteman). The DCM phase was evaporated at 55 to 60 °C on a water bath to obtain a crude bio-oil.

The electrical conductivity (EC) and the pH of aqueous phase were measured by desk type meters (F-70/

Table 1 Analysis results of algae powder

Characteristics	wt%
Moisture	5.6 %
Volatile	65.4 %
Fixed carbon	2.7 %
Ash content	26.3 %
Lipid content	22.6 %
C	38.3 %
H	5.0 %
O (by calculating)	51.0 %
N	4.5 %
P	0.48 %
K	0.41 %
S	0.36 %

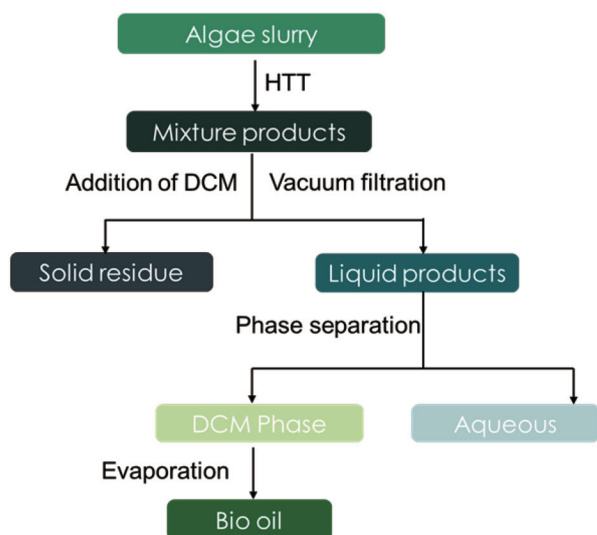


Fig. 1 Products separation procedure

DS-70 series, Laqua Inc.). And the $\text{NH}_4^+\text{-N}$ were determined by the way of the Kjeldahl steam distillation. The macro nutrient element and trace element measurement methods were same as those for the feedstock, but without digestion. The aqueous phase was weighed right after the separation. The mass of aqueous products was defined as aqueous yield. The aqueous recovery and element recovery in this study is defined as:

Aqueous recovery

$$= (\text{Mass of aqueous products} / \text{Mass of water added}) \times 100$$

Element recovery of aqueous phase

$$= (\text{Mass of the element in aqueous phase products} / \text{Mass of the element in algae}) \times 100$$

3. Results and Discussion

3.1 Effects of algae loading rate

The hydrothermal treatment with different algae loading rates resulted in different yields of the aqueous products as shown in Fig. 2. The gravimetric yields of the aqueous products gradually decreased from 84.6 to 58.1 g with the decrease of water addition in the total 100 g feedstock. The aqueous recovery is around 88 % because some part of the water was lost due to the residual steam discharge, the water content in oil and solid products, as well as the consumption in the hydrolysis reaction. As described by Kumar¹¹⁾, water as a reactant leads to the hydrolysis reaction and a rapid degradation of the polymeric structure of biomass into water. The EC values shown in Fig. 3 exhibited a linear correlation with the algae loading rate, and the calculated Pearson's r correlation coefficient was 0.94. It could be inferred that the organic compounds released from algae cells were decomposed and dissolved into soluble small organic molecules or metals¹²⁾.

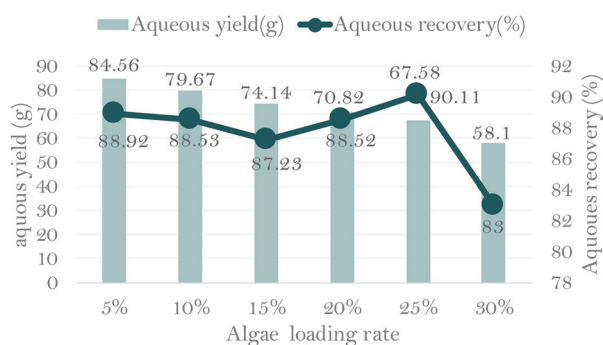


Fig. 2 Aqueous yield and recovery affected by the algae loading rate

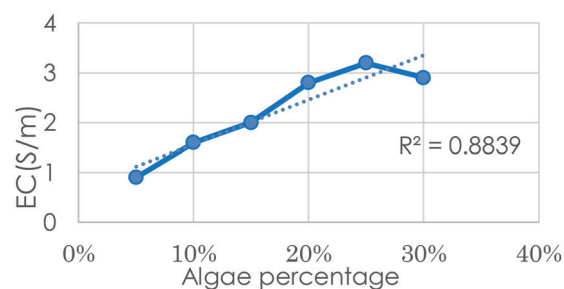


Fig. 3 EC value in aqueous products

The nutrient analysis without dilution are listed in Table 2 with a typical growth media 3N-BBM+V¹³⁾ (Bold Basal Medium with 3-fold Nitrogen and Vitamins) to compare the levels of major nutritious elements. The concentration of TC, TN and $\text{NH}_4^+\text{-N}$, K, Mg, Na are very high, orders of magnitude higher than those found in the media even in the case of 5% algae loading rate. So is the $\text{NH}_4^+\text{-N}$, which the microorganism can use directly. As for the trace elements, there were Si, B, Fe and Mn over 0.1 ppm, which did not reach the level to threaten the growth of algae.

By increasing the algae loading rate, the concentration of nutrient TC, TN, $\text{NH}_4^+\text{-N}$, K and many other trace elements in the aqueous products increased remarkably. The increase of the algae loading rate leads to more algae protein, lipid or polysaccharide hydrolyzed and into the hydrophilic amino acid, organic acid derivatives or monosaccharide, and further degradation during the hydrothermal treatment¹⁴⁾. However, the P could not reach the standard. It increased when the pH is lower than 7, then decreased when in alkaline. According to Peter. J⁵⁾, most of P in aqueous phase is present as free phosphate (as their measurement for total phosphorus and phosphate were similar), which can easily precipitate with some metals contained in algae, such as Ca, Mg, Fe. It could be confirmed by the fact that, with the comparable amount of Mg in the parent feedstock, the Ca was not detectable in

Table 2 The effects of algae loading rate on nutrition elements of aqueous products compared with 3N-BBM+V

(ppm)	3N-BBM+V	5%	10%	15%
pH	6.8	6.65	6.72	6.87
TC ^{*1}	–	10053	16544	22997
TN ^{*2}	671	2111	4687	6224
NH ₄ ⁺ -N	–	1705	3182	4548
NO ₃ ⁻ -N	547	–	–	–
P	51	15.4	21.3	40.8
K	63	207.5	414.1	613.2
S	98	75.7	142.5	221.9
Na	77	65	120	170
Mg	7.3	25	52	83
Ca	6.8	NA	NA	NA
Si	–	8.2	8.4	7.3
B	–	0.72	1.4	2.0
Fe	1.03	0.28	0.74	1.1
Mn	11.6	0.13	0.22	0.36
(ppm)	3N-BBM+V	20%	25%	30%
pH	6.8	7.08	7.39	7.89
TC ^{*1}	–	32992	42011	51414
TN ^{*2}	671	8527	10426	12549
NH ₄ ⁺ -N	–	6540	7505	9360
NO ₃ ⁻ -N	547	–	–	–
P	51	22.9	17.4	7.2
K	63	851.8	1139.3	1315.1
S	98	266.1	343.7	334.3
Na	77	240	320	380
Mg	7.3	96	110	120
Ca	6.8	NA	NA	NA
Si	–	9.4	6.8	4.8
B	–	2.8	3.5	3.9
Fe	1.03	1.6	2.5	2.3
Mn	11.6	0.49	0.71	0.96

■ means the values higher than 3N-BBM+V, ■ means the values lower than 3N-BBM+V

TC^{*1} = Total carbon, TN^{*2} = Total nitrogen

the aqueous product. Meanwhile, there are some phosphates which become deposits or has lower solubility in alkaline environment, such as magnesium ammonium phosphate (MgNH₄PO₄).

In conclusion, it is satisfying to find that the levels of many necessary nutrition in the aqueous products were acceptable as the algae cultivation medium. However, the macronutrient amount like TC and TN at 30 % algae loading rate, was not as high as 6 times of those at 5 % algae loading rate. Thereby, lower

algae loading rates are more economic, as more nutrient could be recovered to the water phase. The reason includes, firstly, the decreasing water amount gave lower water soluble products per mass of algae feedstock, and secondly, the alkaline environment in higher algae loading rate made P recovery very low.

3.2 Effects of the treatment temperature

Although the aqueous products obtained from a higher concentration of algae showed a high level of nutrition content, their recovery rates were lower than in the case of a lower concentration of algae. Combined with the bio-oil analysis, 10 % algae loading rate gave relatively high yield of two target products, bio-oil and water soluble fraction. So it was chosen as the study for effects from hydrothermal temperature. The gravimetric yields of the aqueous products obtained using different heating temperatures were relatively stable in the range of 78.6 g to 83.2 g corresponding to the aqueous recovery of 87.3 % to 92.4 %. The EC values slightly increased with the increase of the temperature, which indicates that the temperature will have a positive influence on the decomposition of algae biomass to the water phase. Different from the products in the algae loading rate series experiments, the color of the aqueous products in the temperature series experiments changed and was getting lighter, from dark brown to bright yellow as the temperature increased.

The elements analysis results (without dilution) compared with typical algal growth medium are summarized in Table 3. For the elemental composition, the major nutrition, TC and TN content showed a high level and firstly increased to the peak at 230 °C then decreased. P and S decreased along with the increasing of the temperature, and became lower than the standard value at 230 °C, 250 °C respectively. K and Na contents were much higher than the standard growth medium, kept at relatively stable values. The trace elements over 0.1 ppm were listed. Obvious decreasing trends were also found in these elements, such as Mg, Si, B, Fe and Mn. Even so, the concentrations of K, Na, Mg were very abundant compared with the culture medium. The pH value of the liquid increased from acid to alkaline.

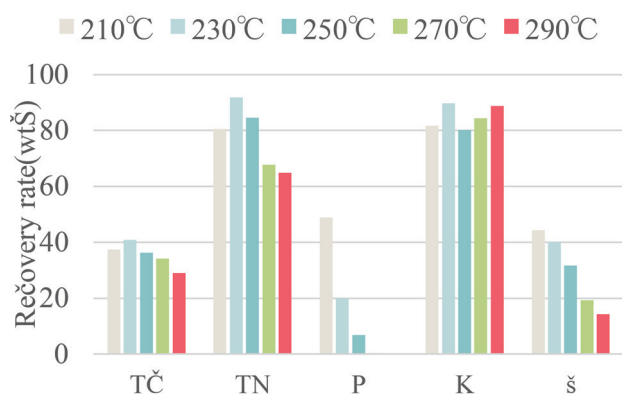
To further understand the elemental distribution affected by the reaction temperature, the element recovery was calculated and shown in Fig. 4. TC and TN in aqueous products showed the same trend as concentrations of them, increased to 230 °C, then decreased. As described by Andrew *et al.*¹⁴⁾, Alba *et al.*¹⁵⁾, and Toor *et al.*¹⁶⁾, the solid algae feedstock was firstly hydrolyzed and liquefied into fragments in oil or water soluble form under a lower heating temperature, while these unstable active parts could polymerize again into solid chars or further degraded to gas as the temperature and the active energy inside the bonds increased. Specifically in this study, the algae proteins, polysaccharides and lipid could be hydrolyzed and depolymerized into the hydrophilic amino acids, monosaccharides and oleophilic organic derivatives, etc. at

Table 3 The effects of temperature on nutrition elements of aqueous products compared with 3N-BBM+V

(ppm)	3N-BBM+V	210 °C	230 °C	250 °C
EC(S/m)		1.45	1.516	1.595
pH	6.8	5.85	6.39	6.77
TC* ¹	–	17192	18793	16211
TN* ²	671	4599	5310	4674
NH ₄ ⁺ -N	–	1694	2529	3180
NO ₃ ⁻ -N	547	–	–	–
P	51	284.7	116.6	21.3
K	63	423	465	416
S	9.8	193	174	145
Na	77	110	120	130
Mg	7.3	97	69	62
Ca	6.8	NA	NA	NA
Si	–	13	8	7.8
B	–	1.6	1.5	1.7
Fe	1.03	2.4	1.2	0.64
Mn	11.6	1.3	0.62	0.28
(ppm)	3N-BBM+V	270 °C	290 °C	
EC(S/m)		1.676	1.779	
pH	6.8	8.2	8.13	
TC* ¹	–	15639	13272	
TN* ²	671	3835	3724	
NH ₄ ⁺ -N	–	3160	3264	
NO ₃ ⁻ -N	547	–	–	
P	51	1.7	1.0	
K	63	438	460	
S	9.8	86	62	
Na	77	130	130	
Mg	7.3	31	2.8	
Ca	6.8	NA	NA	
Si	–	7	5	
B	–	1.4	1.2	
Fe	1.03	0.25	NA	
Mn	11.6	0.18	NA	

■ means the values higher than 3N-BBM+V, ■ means the values lower than 3N-BBM+V

TC*¹ = Total, TN*² = Total nitrogen

**Fig. 4** Element recovery in aqueous products

a low temperature. For the major component TC, it made sense that, these fragments and free radicals increased in subcritical environment, could be further reacted to form char or bio-oil, or degraded through the decarboxylation to leave short intermediates and CO₂ etc.^{10) 14)}. As reported in the previous studies^{17) 18)}, CO₂ was usually the predominant gaseous product at the temperature lower than 350 °C. As for N, a remarkable deamination of amino acid can be inferred from the increasing NH₄⁺-N amount with temperature. So the decrease of TN in aqueous phase is more likely due to the abundant active NH₄⁺-N transferring to oil component, deposit salts or complex in solid residue.

The change of pH value was also consistent with this hypothesis. Some hydrophilic amino acid or short organic acid were produced firstly, which made the aqueous phase acid. Then these organic acid could be further decomposed through the decarboxylation or the deamination reactions, and be left as smaller organic acid, NH₄⁺ and CO₂. Therefore, pH increased with the increased NH₄⁺ and deamination. According to the researches reviewed by Andrew Peterson et al.¹⁴⁾, most optimal yields of amino acid were reported at 250 °C.

Potassium recovery was kept at very high value around 85 %, and it could be released even under a lower temperature. Therefore it could be inferred that K in algae was present in an exchangeable form rather than the bonded form to organic matter. And K is not so easy to become deposits with anions compared with other metals.

The P content together with Mg, Fe, Si and Mn contents showed an apparent decreasing trend with the increased reaction intensity caused by increasing the temperature. Calcium is still barely detected in the aqueous phase. It is possible because that the phosphate radical hydrolyzed from phospholipid, nucleic acid, ATP etc. were precipitated with the above mentioned metal ions such as in the form of Ca₃(PO₄)₂, especially when the aqueous part become alkaline, as discussed before.

The decreasing tendency with the increase of the reaction temperature can be found for the S content in aqueous phase. Although the reaction was remained unclear, this was likely due to the hydrosulphonyl containing amino acid which could be produced by hydrolysis from protein at a lower temperature. However, at a higher temperature, they could also repolymerize to oil and char, or degraded to form deposit as phosphate.

3.3 Evaluation and dilution

The aqueous products from hydrothermal treatment of algae were rich in nutrients for algae growth. Under the condition of the reaction temperature of 230 °C with

the algae loading rate of 10 %, the recovery of TC and TN reached the peak of 38.8 % and 87.2 %, respectively. The K recovery was as high as 89.7 %. A certain amount of P and S recovery were also observed. But the highest oil yield happened at 250 °C. Compared with the aqueous part from 230 °C, much more $\text{NH}_4^+\text{-N}$ could be recovered into the aqueous phase, but also a reduction in P and K might occur. So further dilution should be considered for the aqueous products from these two temperatures at 10 %.

There are researches ^{4) 6)} reporting that a heavy dilution of aqueous products is necessary to avoid the substantial organic substances inhibitor like phenol. But Alba *et al.* ⁷⁾ gave a different view that, the high amount of organic substances toxicity could be excluded as the limiting factors for growth, and the growth reduction was finally considered due to a lack of micronutrient such as Mg. Metals like Ni could also be excluded as the inhibitor as it showed very low amount. The high ammonium (NH_4^+) content could affect the algae growth through the shift to ammonia (NH_3), since it was reported that a certain amount of ammonia could inhibit the growth of a specific algae strain. As the equilibrium between ammonium and ammonia is mainly affected by the pH, the ammonium content was excluded as a factor for dilution in our case since the pH of the aqueous phase of 230 °C and 250 °C are close to 7.

3N-BBM+V is highly enriched of nutrition and used for many of the green algae. The high content of nitrogen is the unique point of this medium. According to the discussion before, the high organic carbon content (TOC), metal content and ammonium were neglected as the consideration. The dilution times (4.6 times for 230 °C/10 %, 5.8 times for 250 °C/10 %) was determined mainly based on the major nutrients N, by making the amount of $\text{NH}_4^+\text{-N}$ in aqueous products equal to $\text{NO}_3\text{-N}$ in standard medium. However, most trace elements appeared to be much higher than the medium. To use the aqueous products more economically and effectively, the macro nutrients should be lowered to comparable level with the standard medium. As the medium is rich in 3 fold of nitrogen content, the aqueous was furthered diluted for another 3 times (13.8 times for 230 °C /10 %, 17.4 times for 250 °C /10 %). The comparison of 3N-BBM+V and further diluted aqueous products is shown in Table 4. The results showed a comparable level of N, S and Mg amount, and lack of P, K, and micronutrients. By using the 13.8 times diluted aqueous products obtained at 230 °C, 100 % of N, S, 16.5 % of P, 53.5 % of K, and part of Na, Mg could be saved. By using the 17.4 times diluted aqueous products obtained at 250 °C, 100 % of N, Na, 84.7 % of S, 37.9 % of K, and a part of P, Mg could be saved.

Table 4 Diluted aqueous products compared to standard media 3N-BBM+V

(ppm)	3N-B BM+V	13.8 dilution 230°C /10%	17.4 dilution 250°C /10%
TC	–	1361.8	931.7
TN	671	384.8	268.6
$\text{NH}_4^+\text{-N}$	–	183.3	182.8
$\text{NO}_3\text{-N}$	547	–	–
P	51	8.4	1.2
K	63	33.7	23.9
S	9.8	12.6	8.3
Na	77	8.7	7.5
Mg	7.3	5.0	3.6
Ca	6.8	NA	NA
Si	–	0.58	0.45
B	–	0.11	0.10
Fe	1.03	0.09	0.04
Mn	11.6	0.04	0.02

■ means the values higher than 3N-BBM+V, ■ means the values lower than 3N-BBM+V

However, as different algae species may have different nutrients requirement and inhibitors, the dilution times and additional nutrients should be considered and adjusted based on the practical cultivation.

5. Conclusion

With the increase of the algae percentage in the feedstock, TC, TN, K and many micronutrients in the aqueous products increased. However, P and some of metals were not affected by the algae loading rate, but showed a stronger relationship with pH. The temperature gave different element release to aqueous phase. TC and TN increased until 230 °C then decreased. The P, S and some metal elements except for Na and K, decreased with the temperature. Na and K concentration were kept at a relatively high and stable level. Another macro nutrient Ca was undetectable in the aqueous products. Additionally, the majority of N, K and Na could be recovered in the aqueous products. A certain amount of P, S, and Mg are also considerable in lower temperatures.

The aqueous products of the condition at 10 % algae loading rate, 230 °C and 250 °C, showing high amounts of C, N and K nutrients and considerable trace elements, demonstrated that the aqueous products from the hydrothermal treatment of algae are rich in nutrients for algae growth and have an opportunity for producing value-added aqueous coproducts.

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