Nutrient bioavailability and uptake by a cyanobacteria consortium cultivated at high pH and alkalinity

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May 5, 2020

Abstract

Alkaliphilic microalgae and cyanobacteria have gained significant importance due to their robustness, high biomass productivity, and ability to efficiently capture carbon dioxide directly from the atmosphere. To grow these alkaliphiles under high pH (pH >10.4) and high alkalinity conditions (0.1 - 0.5 M), substantial amounts of nutrients are required, which could potentially increase the operating costs of cultivation and adversely affect its environmental footprint. One conceivable way of tackling this issue is by re-using the spent medium and supplementing only the depleted nutrients. To effectively re-use the spent medium, first it is important to understand the nutrient bioavailability and uptake by alkaliphiles. In this study, we have determined the bioavailability of nutrients (e.g. C, N, P, S, Mg, S, Ca, Fe, etc.) in a high pH (> 10.4) and alkalinity (0.5 M) medium. Our results show that –with the exception of Mg, Ca, and Fe– all the nutrients are in bioavailable form for microbial growth. The availability of Mg, Ca, and Fe is limited because of precipitate formation with carbonates and hydroxides. Additionally, we have also carried out cultivation experiments to determine biomass productivity, elemental composition, and stoichiometric formula based on nutrient uptake. The cyanobacterial cultures grew well without any inhibition and a maximum productivity of 153 mg-AFDW L^{-1} d^{-1} was achieved. The elemental composition of biomass suggested that Mg and Ca content in biomass is low, consistent with the limited availability of these elements during the growth. Finally, the derived stoichiometric equation resulted in the following chemical formula $CH_{1.75}N_{0.17}O_{0.41}P_{0.003}$.

1. Introduction

Photosynthetic microorganisms such as eukaryotic microalgae and cyanobacteria are a promising source of biomass for the production of bioenergy and bioproducts (Chisti 2007; Khan et al. 2018). These microorganisms can grow on non-arable lands and could potentially enable higher areal productivities than traditional food crops (Brennan 2010; Singh et al. 2017). Eukaryotic microalgae are considered to play a significant role in the renewable energy sector as they have relatively higher lipid content. Whereas, cyanobacteria are currently cultivated for the production of high value products such as pigments, proteins, and vitamins (Singh et al. 2017). The global demand for these high value products is also expected to increase significantly (Bhalamurugan et al. 2018; Singh et al. 2017).

Despite their many advantages, cultivation of these photosynthetic microorganisms is still not sustainable (Khan et al. 2018). These microorganisms require high amounts of macro and micro-nutrients (C, N, P, S, Fe, etc.). When biomass productivity is high, the rate of nutrient uptake would also be high, which in turn could lead to increased nutrient demand (Acién Fernández et al. 2018; Barbera et al. 2018). This could potentially compete in regards with the nutrient supply (i.e. fertilizer) for the agricultural crops (Markou et al. 2014).

To mitigate the high nutrient demand, various nutrient recycling strategies have been proposed (Acién Fernández et al. 2018; Barbera et al. 2018; Farooq et al. 2015). Two such strategies are: 1) usage of wastewater as a main source of nutrients and 2) re-use of spent media with supplementation of the depleted nutrients (Acién Fernández et al. 2012; Acién Fernández et al. 2018; Barbera et al. 2018). Though usage of wastewater appears to be a promising strategy, it could potentially introduce foreign substances (e.g. heavy metal ions, pathogenic microbes, etc.) into the algal cultivation system. Therefore, it might not be a viable option for algae that are cultivated to meet food and pharmaceuticals demand (Barbera et al. 2018). On the other hand, the re-use of spent media would definitely mitigate the high nutrient demand while maintaining the biomass quality (Farooq et al. 2015).

However, it is imperative to understand the biology and nutrient uptake capabilities of the microalgal/cyanobacterial cultivation systems to economically recycle the spent medium. For instance, one of the significant macronutrients that needs to be replenished in the spent medium is inorganic carbon (Fields et al. 2014). In cultivation systems that operate at circumneutral pH conditions (pH ~7), depleted inorganic carbon is frequently provided by sparging a CO_2 rich gaseous stream into the spent medium (Fields et al. 2014). However, CO_2 sparging is an inefficient process that requires high energy inputs (Liu et al. 2013). This would increase the overall operating costs. An alternative solution is to use microalgae/cyanobacteria that grow over a pH range of 8.0 – 9.0 where bicarbonate (i.e. the predominant inorganic carbon species) is up-taken via a carbon concentrating mechanism (Ataeian et al. 2019; Raven 1994). Bicarbonate has higher solubility in the growth medium than carbon dioxide (Markou et al. 2014). Unfortunately, the costs associated with supplementing the depleted bicarbonate reserves in the culture medium has been estimated to be three times higher than CO_2sparging (Markou et al. 2014).

Recent findings suggest that the costs associated with CO_2 supply can be minimized or completely eliminated by cultivating microalgae at high pH and alkaline conditions (pH $>$ 10, alkalinity $>$ 0.1 M) (Vadlamani et al. 2019; Vadlamani et al. 2017). In our previous studies, we have demonstrated that cultivation of an alkaliphilic cyanobacterial consortium at high pH (~10.5) and high alkalinity (0.5 M) conditions enabled effective regeneration of inorganic carbon by direct CO_2 capture from air (Ataeian et al. 2019; Sharp et al. 2017). Nonetheless, a clear understanding of nutrients solubility and uptake rates at high pH growth conditions is still required for proper design of the re-cycling spent medium and other downstream processes.

In this study, we have carried out a comprehensive analysis on nutrient solubility/availability and nutrient uptake rates during cultivation of an alkaliphilic cyanobacterial consortium. The outputs of this study would provide insights into 1) bioavailability of the provided nutrients at high pH growth conditions, 2) nutrient uptake rates and 3) available nutrient at the end of a cultivation cycle. We have also determined the element composition of the obtained biomass.

2. Materials and Methods

2.1 Cultivation conditions

A laboratory grown microbial consortium dominated by cyanobacteria was used in all experiments (Ataeian et al. 2019). This consortium was originally developed from photosynthetically active microbial mats obtained from soda lakes located in the Cariboo Plateau (British Columbia, Canada) (Sharp et al. 2017). A synthetic growth medium was formulated to simulate the high pH and alkalinity conditions dominant in the soda lakes. This medium contained the following: Na_2CO_3 (210.98 mM), $NaHCO_3$ (77.85 mM), $NaNO_3$ (3.06 mM), NH_4Cl (0.92 mM), KH_2PO_4, (1.44 mM), $MgSO_4{\cdot}7H_2O$ (1 mM), $CaCl_2{\cdot}2H_2O$ (0.17 mM), NaCl (0.43 mM), KCl (6.04 mM) $FeCl_3{\cdot}6H_2O$ (0.04 mM) and 300 µL/L of a trace metal solution. The trace metal solution comprised of H_3BO_3 (9.7 mM), $MnCl_2{\cdot}4H_2O$ (1.26 mM), $ZnCl_2$ anhydrous (0.15 mM), $CuCl_2{\cdot}2H_2O$ (0.11 mM), Na2MoO4·2H2O (0.07 mM), CoCl2·6H2O (0.06 mM), $NiCl_2{\cdot}6H2O$ (0.04 mM), KBr (0.08 mM) (Ataeian et al. 2019; Vadlamani et al. 2017). Incubations were performed at room temperature (20°C) in 500 mL Erlenmeyer flasks with a working volume of 300 mL. Cultures were inoculated at an initial

biomass concentration of 0.4 g-AFDW/L. These cultures were mixed at a constant speed (100 rpm) using a shaker incubator (Thermo Scientific, MaxQ HP). Full spectrum LED lights (Model T5H0; 6400K, Sunblaster Holdings ULC, Langley, BC, Canada) were used to provide a light intensity of 200 μmol photons·$m^{-2}s^{-1}$ and a light:dark cycle of 16:8 hr was applied (Ataeian et al. 2019). All experiments were carried out for four days in triplicates.

2.2 Analytical methods

2.2.1 Supernatant analysis

The pH of the culture samples obtained during the incubation was measured using a pH meter (Seven Compact^{TM} S220, Mettler Toledo, USA). The cultures were then centrifuged for 10 minutes at 3900g (Allegra X-22R, Beckman Coulter, USA). The supernatant obtained after centrifugation was analysed for total alkalinity (TA), anion and cation concentrations, ammonia concentration, and total dissolved nitrogen concentration. In brief, TA was measured using a G20 compact titrator (Mettler Toledo, USA); 40 mL of supernatant was taken in a beaker and titrated with 0.2N H_2SO_4 until the samples reached an end-point of pH = 4.3 (Vadlamani et al. 2019). Bicarbonate and carbonate concentrations were estimated using the measure pH and TA (Vadlamani et al. 2019; Vadlamani et al. 2017).

Anion (NO_3^-, PO_4^{3-}, SO_4^{2-}) depletion rates were measured using an ion chromatograph equipped with an IonPac AS18 anion and a conductivity detector (DIONEX ICS 2000, Thermo Fisher, USA) (Vadlamani et al. 2017). Ammonium concentration was determined by following the procedure described by Sims et al. (Sims et al. 1995). Total nitrogen concentration was assessed using a previously described scaled down version of the Persulfate Digestion method (Hach Method 10071, Hach, USA) (Vadlamani et al. 2017).

2.2.2 X-ray mapping of precipitates

To determine the chemical nature of precipitates in the growth medium, we used an FEI Quanta FEG 250 environmental field emission scanning electron microscope (E-FESEM) in combination with a Bruker QUANTAX Energy Dispersive X-ray Spectroscopy (EDS) system. In brief, freshly prepared medium was filtered using 0.2 μm mixed cellulose esters (MCE) membrane filter to separate the precipitates. The filter membrane containing the precipitates was then air dried for 24 hr. A small portion of the dried filter membrane was then mounted onto stubs using double sided carbon tape without any surface modifications. The stubs containing the filter membrane were then analyzed to obtain the elemental maps using E-FESEM/EDS system (operated at primary energy – 15KeV). These elemental maps were then used to infer the chemical composition of the precipitates.

2.2.3 Quantification of cations

Sodium, potassium, phosphorous, sulfur, magnesium calcium, and iron were analyzed on both freshly prepared medium and supernantant obtained after growth using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS, Xseries 2, Thermo Scientific, USA) (Hanifzadeh et al. 2018). While the freshly prepared medium was analyzed at both pH 10.46 (initial pH of growth medium) and reduced pH (pH <3, reduced using 3% HNO_3), supernatant obtained after each day of incubation was analyzed only at reduced pH. Cation concentrations were estimated from a calibration curve generated by applying the same protocol as samples to standards.

2.2.4 Visiual MINTEQ modelling

Visual MINTEQ was used to estimate ion speciation in our high pH and alkaline medium. The chemical equilibrium model Visual Minteq 3.1 (https://vminteq.lwr.kth.se/) is based on the program PC MINTEQA2 (Allison et al. 1991). Computations were carried out at the following fixed parameters: Temperature - 20 °C, pH - 10.46 and ionic strength - 0.73.

2.2.5 Biomass productivity

The wet biomass obtained after centrifugation was frozen at -80°C overnight and then freeze dried at -50°C and at a pressure of 1 mPa using a bench top freeze dryer (Labconco, Kansas city, MO) (Vadlamani et al. 2019). Ash content of the freeze-dried biomass was then analysed using a previously reported National Renewable Energy Laboratories (NREL) method (Sluiter et al. 2008). Ash content obtained was then used to estimate the biomass concentration and productivity using the relationships provided in the supplementary information.

2.2.6 Biomass composition analysis

The carbon, nitrogen, and hydrogen content of the freeze-dried biomass was determined as previously described (Ataeian et al. 2019). The content of other elements in the biomass was determined by ICP-MS analysis of the digested biomass. First, digestion of the biomass was carried out using a MARS 6 – Microwave Digestion System (CEM Corporation, USA) (Hanifzadeh et al. 2018). In brief, 10 mL of HNO_3 were added to about 100 mg of freeze-dried biomass and digested at 250°C and at a pressure of 55.16 bar for 30 minutes. These samples were then diluted with Milli-Q water (to fit in the calibration range) and analyzed for metallic (Na, Mg, Ca, K, Fe, Co, Cu, Ni, Mn, and Zn) and some non-metallic (P, S and B) elements using an ICP-MS (instrument company name) (Hanifzadeh et al. 2018) The measured elemental concentrations were then used to estimate the uptake rate.

3. Results and Discussion

3.1 Bioavailability of nutrients

Bioavailability is defined as the soluble nutrients available for uptake by microalgae and/or cyanobacteria during the growth (Lee et al. 2009; Suzuki et al. 1995). Because the pH (>10.4) and ionic strength (0.73 M) of our growth medium are high, we suspected that some elements (e.g. Mg, Ca, Fe, etc.) would precipitate (e.g. $CaCO_3$, $MgCO_3$, $Fe(OH)_3$etc.) and might not be completely bioavailable (Vandamme et al. 2012). However, in our previously reported study (Ataeian et al. 2019), we observed that the cyanobacterial consortium enriched from Soda Lake microbial mats (Cariboo, BC) grew well when cultivated over a pH range of 10.4 – 11.2 at a total alkalinity of 0.5 M. This suggested that sufficient nutrients were bioavailable to support the growth. Nonetheless, it is important to determine the type of precipitates formed and quantify them. In this study, we performed a comprehensive analysis on the freshly prepared medium to determine the bioavailability of each element during the cultivation.

First, we used Visual Minteq 3.1 software (KTH, Sweden) to predict both free ion form concentrations and precipitates in our high pH and high alkalinity medium. It was predicted that the solubility products for Mg^{2+}, Ca^{2+}, and Fe^{3+} species were the lowest of all the free-ion species at high pH. Mg and Ca were expected to precipitate as carbonates and Fe as hydroxide. Following the model predictions, we have collected precipitates from freshly prepared medium (see description in section 2.2.2) and analyzed them by SEM-EDS. The SEM images (Figures 1 a, b and 2 a, b) show that the surface of the filter was covered with amorphous precipitates. Further, EDS analysis (Figure 1c and 2c) confirmed that the amorphous precipitates formed were in fact $CaCO_3$ and $Fe(OH)_3$.

Finally, culture medium at a pH = 10.46 was analyzed on ICP-MS to determine the bioavailability of sodium, potassium, phosphorous, sulfur, magnesium calcium, and iron. In parallel, the pH of the same culture medium was reduced by acidification until pH < 3 and was also analyzed for these elements. The data obtained from the two analyses were compared with the concentrations actually provided in the growth medium (See Table 1). The concentrations of sodium, potassium, phosphorous, and sulfur were similar in both analyses and are comparable to the amounts given in the medium (Table 1). However, magnesium, calcium, and iron were at concentrations significantly lower in the high pH medium when compared to those after the pH was lowered

(Table 1). These elements might have reacted with other ions (e.g. CO_3^{2-} and OH^-) resulting in the formation of precipitates. Overall, the experimental data are in agreement with model predictions and indicate that bioavailability of Mg, Ca and Fe was limited due to precipitation.

From our previously reported study (Ataeian et al. 2019), it is clear that over a pH range of 8-9.5, dissolved CO_2 is exceedingly small and HCO_3^- becomes the predominant species. As the pH increases (pH > 10), CO_3^{2-} becomes dominant. Since cyanobacteria have ability to utilize both CO_2 and HCO_3^-, but not CO_3^{2-} (Raven 1994), it was important to determine the bicarbonate concentration in the high pH medium. The availability of HCO_3^- was estimated using the measured TA (0.5 ± 0.003 M) and pH (10.46 ± 0.02) values (Vadlamani et al. 2019; Vadlamani et al. 2017). It was observed that the estimated HCO_3^- reserve (33.3 ± 0.8 mM) was lower than the actual concentration provided in the growth medium, as shown in Table 1. This suggested that a portion of the overall HCO_3^- provided is converted into CO_3^{2-} due to pH dependant inorganic carbon speciation (Wolf-Gladrow et al. 2007).

Nitrogen, another important macronutrient, was also analysed for its bioavailability. We supplied an overall nitrogen content of 3.98 mM in the form of NO_3^- (3.06 mM) and NH_4^+ (0.92 mM). Our NO_3^- measurements indicated that close to 100% of the NO_3^- provided is present in the medium (2.97 ± 0.07 mM, Table 1). On the other hand, measured NH_4^+ concentrations indicated that the medium has 20% lower ammonium content (0.75 ± 0.04 mM, Table 1). The decrease in the N-NH_4^+ could be due to volatile NH_3 formation at high pH conditions (Körner et al. 2001). Overall, more than 90% (3.72 mM) of the nitrogen provided was available in the media for the cyanobacterial growth.

3.2 Biomass growth, carbon, nitrogen, and phosphorous uptake

3.2.1 Biomass growth

Next, we performed cultivation experiments to determine the effect of bioavailable nutrients on productivity and nutrient content of the biomass. The cyanobacterial cultures were inoculated at an initial concentration of 0.43 ± 0.10 g-AFDW/L (Day 0, Figure 1a) to a growth medium at an initial TA and pH of 0.5 ± 0.003 M and 10.46 ± 0.02 respectively (Day 0, Figure 1b). Culture concentration (Figure 1a) and nutrient utilization (Figures 2&3) were monitored over an incubation period of 4 days. Figure 1a shows that the cultures grew well without any apparent growth inhibition to a final biomass concentration of 1.04 g AFDW/L ± 0.12 (Day 4, Figure 1a). The corresponding biomass productivity (0.15 g-AFDW/L/d, estimated using Eq. 3) was higher than previously reported (0.048 g-AFDW/L/d) (Vadlamani et al. 2017).

3.2.2 Carbon

Microalgae and cyanobacteria that grow at high pH have evolved a carbon concentrating mechanism to transport bicarbonate into their cells. The transported bicarbonate is then converted into carbon dioxide by carbonic anhydrase and is assimilated by RuBisCO enzyme (Raven 1994). The overall process results in the release of hydroxyl ions into the culture medium and thereby increases the pH (Raven 1994). In our experiment, the pH increased to 10.69 ± 0.09 by day 4 (Figure 2b) suggesting that the available bicarbonate was indeed utilized during the growth.

The estimated bicarbonate depletion observed at the end of the growth period was 16.4 ± 1.4 mM (Figure 3a). Simultaneously, an increase in carbonate concentration was also observed (8.1 ± 0.18 mM, Figure 3a). Since, for every one mole of carbon fixed, two moles of bicarbonate are converted and one mole of carbonate produced (Ataeian et al. 2019), we can safely assume that the remainder of the bicarbonate (8.4 ± 1.4 mM) was used by the cyanobacterial consortium for growth. However, the net increase in organic carbon accumulated in the biomass (estimated from CHN analysis and ash free dry biomass) was 25.4 ± 4.5 mM (Figure 3a). The net increase in organic carbon content is more than twice the amount of inorganic carbon taken up by cyanobacteria during the growth period. The additional carbon assimilated could be explained by capture of additional CO_2 from the air during cultivation (Ataeian et al. 2019; Vadlamani et al. 2019; Vadlamani et al. 2017).

3.2.3 Nitrogen and Phosphorus

Uptake and utilization rates of nitrogen and phosphorous were analyzed along with carbon (See Figures 3b and 3c). Figure 3b shows, that the initial NO_3^- concentration was 2.97 ± 0.07 and the initial NH_4^+concentration was 0.75 ± 0.04 mM. Total nitrogen depleted at a rate of 1.19 mM/d and at the end of the incubation, almost all the nitrogen supplied in the medium was used. Concomitantly the amount of organic nitrogen in the biomass increased (4.15 ± 0.03 mM, Day 4, Figure 3b). Interestingly, it appeared that there was no significant increase in the biomass during the first three days of incubation (Figure 2a), while 50% of the nitrogen supplied was consumed during this period (Figure 3b).

The initial phosphorous concentration was 1.32 ± 0.04 mM (Day 0, Figure 3c). As the incubation progressed, part of this phosphorous was assimilated. By the end of day 4, nearly 0.14 ± 0.007 mM (Day 4, Figure 3c) of the phosphorous was depleted and concomitantly, the amount of phosphorous in the biomass increased by 0.25 ± 0.10 mM.

3.3 Macro (Ca, K, Mg, Fe, S, P and Na) and micro (Co, Cu, Zn, Mn, and Ni) nutrient utilization and up take rates during the growth of cyanobacterial consortium.

3.3.1 Sulfur

For microalgae and cyanobacteria, an important macronutrient is sulfur, found in amino acids (cysteine and methionine), the lipid bilayer of the cell membrane, regulatory compounds, and vitamins (Abdelaziz et al. 2013; Giordano and Prioretti 2016; Markou et al. 2014). Sulfur is typically taken up in the form of sulphate (SO_4^{-2}) and for this reason we provided $MgSO_4$ in our high pH and alkalinity medium. From Figure 4f, we observed that the sulfur concentration in the medium decreased at a rate of 0.04 ± 0.01mM/day while the uptake rate was estimated to be 0.05 ± 0.008 mM/day. The sulfur content of the biomass was in the range of 0.5-0.8 (% (w/w)), within the previously reported range (0.15-1.6%) [8, 26, 28].

3.3.2 Sodium and Potassium

Sodium and potassium play a key role in the growth of microalgae and are involved in protein synthesis, osmotic regulation, and are cofactors for several enzymes (Anh et al. 2004). The potassium uptake rate was estimated to be 0.12 ± 0.01 mM/day and the rate of depletion in the media was 0.2 ± 0.12 mM/day over three days (Figure 4d). Potassium content in the biomass was in the range of 0.7-1.8 (% (w/w)), comparable to previously reported values (Grobbelaar 2004; Silva et al. 2015; Tibbetts et al. 2015; Tokuşoglu and Üunal 2003). Similarly, the uptake rate of sodium was estimated to be 0.86 ± 0.06 mM/day over four days (Figure 4b) and the overall sodium content in the biomass was 1.5-7.5 (% (w/w)).

3.3.3 Magnesium

Magnesium, the central atom of chlorophyll and also crucial for the activation of RuBisCO and several other major enzymes (Ben Amor-Ben Ayed et al. 2016; Hanifzadeh et al. 2018; Markou et al. 2014), typically constitutes between 0.35 - 0.7 (% (w/w)) of the total biomass content (Markou et al. 2014). In this study, the uptake rate of magnesium in the biomass increased at rate of 0.04 ± 0.003 mM/day (Figure 4e) and constituted 0.35-0.46 (% (w/w)) of the biomass. Though the bioavailablity of Mg was limited because of precipitation (see section 3.1), the Mg content is still in the typical range when compared to previously reported values (Ben Amor-Ben Ayed et al. 2016; Hanifzadeh et al. 2018).

3.3.4 Calcium

Another important element in microalgal growth is calcium. Calcium plays a key role in the signal transduction and regulation of enzyme activities in microalgae (Anh et al. 2004). The depletion rate of calcium in the media over 4 days was 0.012 ± 0.003 mM/day which was identical to the estimated uptake rate of 0.012

± 0.003 mM/day (Figure 3c). Calcium content in the biomass was between 0.15 - 0.35 (% (w/w)), which was at the lower end of the reported range in the literature (0.09-3.0%) (Campanella et al. 1998; Tibbetts et al. 2015; Tokuşoglu and Üunal 2003). This could be due to the limited bioavailability of Ca because of the precipitate formation.

3.3.5 Iron

One of the most important elements in photosynthesis is iron, since it plays a key role in electron transfer during photosynthesis and respiration [35, 36]. Typically, iron makes up 0.02-0.7 (% (w/w)) of the total biomass content, which matched up with the results from this study where the iron was found to represent 0.3-0.6 (% (w/w)) of the total biomass. The depletion rate of iron was 0.002 mM/day in the media. Theoretically, depletion rate of iron in the growth medium should match with the uptake rate in the biomass. However, our estimates suggested that the iron content in the biomass is three-fold higher (0.007 ± 0.004mM/day, Figure 4a). The increased iron content could be attributed to the iron precipitates that were formed and trapped in the extracellular polymeric layer surrounding the cells at high pH growth conditions.

3.3.6 Micronutrients (Co, Cu, Zn, Mn, and Ni)

Apart from macronutrients, micronutrients (e.g. Zn, Mn, Co, Cu, etc.) are also important for the microalgal growth. The concentration of the supplied micronutrients was measured as described in Section 2.2.3 and the results are reported in Supplementary Figure 1a-f. The concentration of zinc, a fundamental element for the function of carbonic anhydrase (Blindauer 2008), increased at a rate of 1.19×10^{-4}-04 µM/day in the biomass and the overall zinc content was in the range of 0.0040-0.011(% (w/w)) (Supplementary information, Figure 1e). Manganese made up a similar percentage of the biomass in the range of 0.008-0.03 (% (w/w)), and the uptake rate was estimated to be 6.83×10^{-5}µM/day (Supplementary information, Figure 1a). Nickel, copper, and cobalt made up a similar biomass 0.0008-0.002 (% (w/w)), 0.0015-0.0065 (% (w/w)), and 0.0003-0.0015 (% (w/w)), respectively. Copper had an uptake rate (1.14×10^{-5}µM/day) higher than nickel (2.39×10^{-6} µM/day) and cobalt (3.72×10^{-7} µM/day) (Supplementary information, Figure 1b-d). The biomass content of zinc, copper, cobalt, nickel, and manganese was comparable to what was previously reported in the literature (Grobbelaar 2004; Silva et al. 2015; Tibbetts et al. 2015; Tokuşoglu and Üunal 2003).

3.3.7 Elemental composition and stoichiometric formula of alkaline biomass

Using the experimental data obtained in this study, we estimated the elemental composition of the cyanobacterial consortium. For comparison, we also analyzed the elemental composition of microbial mats collected from four different Soda Lakes located in the Cariboo Plateau, British Columbia, Canada; namely, Last Chance Lake (LCL-M), Probe Lake (PL-M), Deer Lake (DL-M), and Goodenough Lake(GEL-M)]. These mats were used as inoculum for the original enrichment of the consortium (Sharp et al. 2017). Table 2 shows the elemental composition of the mats collected from these lakes. It was observed that the nitrogen and phosphorous content of the microbial mats were lower than the values measured in our experiments, suggesting that these lakes had low nitrogen and phosphorous reserves. Overall, the elemental composition obtained for both the cyanobacterial consortium cultured in this study and the microbial mats collected from the Soda Lakes were comparable to previously reported literature values (Campanella et al. 1998; Silva et al. 2015; Tibbetts et al. 2015; Volkman and Brown 2006).

In addition to the elemental composition reported in Table 2, we have also derived a stoichiometric formula for the alkaline biomass. This formula was estimated to be $CH_{1.75}N_{0.17}O_{0.41}P_{0.003}$(results for all elements shown in Supplementary Table 1) and is similar to the previously reported stoichiometric equation for eukaryotic microalgae ($CH_{1.7}N_{0.14}O_{0.4}P_{0.009}$, (Oswald 1988).

4. Conclusion

In this study it was demonstrated that cyanobacterial cultures collected from Soda Lakes, Cariboo, BC grew well over a pH range of 10.46 – 10.7. Detailed chemical analysis of the biomass and spent culture media indicated that all major nutrients (except Mg, Ca, and Fe) were in bioavailable form during the cultivation of the cyanobacterial consortium under these high pH and high alkalinity growth conditions. Except for carbon, nitrogen, and iron, all other nutrients were sufficiently present in the spent medium at the end of growth cycle. While depleted inorganic carbon can be replenished via carbon dioxide capture either from flue gas or directly from the atmosphere, nitrogen and iron need to be replenished by the addition of an external fertilizer or if possible by recirculation of a recovered stream after downstream processing of the microalgal biomass. The elemental composition of the biomass was estimated from the experimental data. Overall, this information can be used in the design of a strategy for medium reuse and fresh nutrient minimization.

5. Acknowledgements

The authors declare no conflict of interest. The authors are thankful for the assistance provided by Mr. Michael Nightingale and Dr. Bernhard Mayer in the use of Ion Chromatographer and G20 compact titrator; Mr. Brian Ballie for running the samples on ICP-MS; Dr. Pedro Pereira-Almao with the use of the MARS 6 microwave digester; and Christopher Debuhr for letting us use their facility for E-FESEM/EDS system. This research was undertaken thanks in part to funding from the Canada First Research Excellence Fund (CFREF), Alberta Innovates, the Government of Alberta, and the University of Calgary.

6. Supplementary Information

Supplementary information contains relationships to estimate biomass concentration and productivity on ash free dry weight basis. A relationship to estimate the nutrient uptake rate. A figure showing net increase in concentrations of assimilated micronutrients. A figure showing SEM image and EDS spectrum of blank filter paper and a table comparing the empirical formulas of microbial mats collected from Soda Lakes and cyanobacterial consortium cultured in this study.

7. Authors Contributions

Alexander Paquette (AP), Agasteswar Vadlamani (AV), Marc Strous (MS) and Hector De la Hoz Siegler (HS) conceived and planned the experiments. AP and AV carried out the experiments. AP and AV contributed to sample preparation. AP, AV, MS and HS contributed to the interpretation of the results. AP and AV took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis, and final version of the manuscript.

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9. Tables

Table 1: Concentration (mM) of nutrients at high pH (10.4), reduced pH (<3), and bioavailability (%) of nutrients.

Elements	Conc Grow
C-HCO_3^- [c]	33.3
N-NO_3^- [d]	2.97
N-NH_4^+ [e]	0.75
Na	497.6
Mg	0.3 ±
K	8.45
P	1.4 ±
S	0.96
Ca	0.09
Fe	0.005

[a] pH of growth medium was reduced using 5% HNO_3 and analysed on ICP-MS to obtain concentration of elements.
[b] High pH growth medium was directly analysed on ICP-MS to obtain concentration of elements.
[c] (bi)carbonate was using measured TA and pH values.
[d] Nitrate was measured using an ion chromatograph
[e] Ammonium concentrations were determined using a colorimetric method

[a] pH
[b] Hig
[c] (bi)
[d] Nit
[e] Am

Table 2: Comparison between elements in cultivated cyanobacteria consortium, microbial mats from soda lakes (Cariboo Plateau, British Columbia) and literature values.

Elements
C (g/kg)
H (g/kg)
N (g/kg)
Ca (g/kg)
K (g/kg)
Mg (g/kg)
Na (g/kg)
P (g/kg)
S (g/kg)
Cu (mg/kg)
Mn (mg/kg)
Zn (mg/kg)
Ni (mg/kg)
Co (mg/kg)
Fe (mg/kg)

Deer Lake Microbial Mat (DL-M), Probe Lake Microbial Mat (PL-M), Goodenough Lake Microbial Mat (GEL-M) and Last
From Silva et al [34], Tibbets et al [31], Campanella et al [36], Volkman and Brown et al [39]

10. Figures

Figure 1: SEM image and EDS spectrum of the recovered precipitates a) CaCO3 and b) Fe(OH)3.

Figure 2: (a) Increase in the biomass concentration and (b) change in pH (left y-axis) and total alkalinity (right y-axis) over the incubation period. Error bars represent the standard deviation of the triplicate samples for each time point.

Figure 3: (a) Concentration of bicarbonate (squares, left y-axis) and carbonate (circles, left y-axis) in the media and change in carbon in the biomass (mM) (triangles, right y-axis). (b) Soluble nitrogen (NH4+ and NO3-) and (c) phosphorus depleted in the media and change in biomass. Values shown in the graphs are averages based on three replicates. Error bars represent the standard deviation from the three replicates.

Figure 4: Concentration of iron (a), sodium (b), calcium (c), potassium (d), magnesium (e), and phosphorus (f) in the spent media and biomass. Values shown in the graphs are averages based on three replicates and error bars represent the standard deviation of the triplicate samples for each time point.