Biological silicon removal from coal seam gas water

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School of Chemical Engineering
**Abstract**

High silica concentrations can cause silica-related fouling in reverse osmosis and heat exchanger water treatment processes. Conventional silica reduction processes to mitigate fouling produce a waste, which must be treated before disposal. This research investigates a waste free biological alternative to conventional silica reduction processes. The biological process utilises the growth of diatoms to convert dissolved silica into amorphous silica, which is the main constituent in the diatom frustule (shell). When removed from the water, the diatom provides a waste free product which contains lipids, protein and carbohydrates which can be used for biofuels and animal feedstock.

The research involved growing diatoms in Coal Seam Gas (CSG) water then removing the diatoms using sieves, thus removing the original dissolved silica as amorphous silica. The research found the growth of both native and introduced diatoms resulted in a reduction of dissolved silica, provided the necessary nutrients were present and biologically available. The diatom growth rate varied from 0.8 to 5.5 g/m².d (specific growth rate: 0.6 to 2.5 g/g.d). Diatom growth led to the removal of dissolved silicon (Si) at a rate of 120-800 mg Si/m².d (or 1.2-8 kg Si/ha.d). The net biomass silicon content was 3 wt%. The lower growth rate occurred when trialling a single diatom species in 150mm deep containers, while the higher growth rate was achieved with multiple species in 300mm deep containers. It is expected diatom growth rate per unit area will increase with deeper containers until light availability constrains growth.

Given these diatom growth and silicon removal rates, 1.5 to 10 ha of ponds per ML of water processed per day would be sufficient to remove all of the silicon from a typical CSG water treatment facility (Si load is typically 12 kg Si/ML); less area would be required to reduce the silicon concentration to manageable levels.

For the biological process to effectively reduce silicon in CSG water the dissolved silicon concentration must be 2 mg/L prior to micro filtration. To achieve 2 mg/L for a typical 10 ML/day RO plant, 10 mg/L or 100 kg/day of silicon must be removed. If the mid range (500 mg Si/m².d) silicon reduction rate achieved in the lab can be achieved on site, a 20 ha pond would be required.
**Declaration by author**

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

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**Publications during candidature**

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**Publications included in this thesis**

No publications
Contributions by others to the thesis
Dr Steven Pratt provided the concept to remove dissolved silica using diatoms and also provided guidance and critical review of the experimental work and thesis. Dr Beatrice Keller-Lehmann, Jianguang Li and Nathan Clayton provided the chemical analysis of wastewater and reactor samples.

Statement of parts of the thesis submitted to qualify for the award of another degree
None
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1. INTRODUCTION

1.1. Background

The water liberated with recovery of coal seam gas (CSG) from coal seams in the Bowen and Surat basins in central and southern Queensland contains a number of impurities including sodium chloride and dissolved silicon. To enable beneficial use of the liberated water (i.e. to enable the water to not be considered a waste product the water is processed by water treatment plants close to the coal seam gas extraction wells.

A typical CSG water treatment process, as shown in Figure 1, treats 10ML/day raw CSG water. The raw CSG water (process point |1|) is discharged into a holding pond and then processed into approximately 2ML/day brine (process point |3|) and 8 ML/day permeate (process point |5|). The brine is further concentrated in evaporation ponds and then is processed through the heat exchangers/brine concentrators (process point |4|) to produce highly concentrated brine. The concentrated brine is held in storage for further treatment or disposal.

Reverse osmosis (RO) is the primary water treatment process used by the CSG companies to remove salts and other impurities. The RO process produces permeate and concentrate/brine (also described as RO reject). The permeate water can be used for agriculture, and/or injected back into the aquifers and/or other beneficial purposes. The concentrate/ brine is considered a waste product. Preventing this brine from polluting the environment is an important responsibility of CSG producers. Several CSG producers in Australia are considering a zero waste-liquid discharge (ZWLD) strategy for sustainable management of CSG brine. According to the ZWLD strategy, the brine is further concentrated and finally recovered as dry salts.
Concentration of the brine, post the evaporation ponds, involves evaporation of water using a series of heat exchangers (refer Figure 2), but the efficiency of this process can be substantially hindered by fouling. Silicon removal can improve the efficiency of these downstream heat exchangers and the upfront RO by reducing fouling. Silica or silicates have been known to foul the RO process if the silicon concentration of the RO reject exceeds 60 mg/L at 25°C [3]. Additionally, when brine is concentrated the dissolved silicon can precipitate and foul the heat exchangers.
In the CSG water treatment process RO silica fouling has not occurred. However, silica fouling of the heat exchangers has occurred despite the increased temperature and pH of the heat exchangers which should increase silicon solubility (SiO₂ solubility is 175 mg/L at 25 °C, 250 mg/L at 50 °C). The fouling is possibly due to silicates reacting with calcium, magnesium, iron, manganese or aluminium to form insoluble polymer silicates [4]. The presence of Al³⁺ and Fe³⁺ ions at concentrations greater than 0.05 mg/L are also likely to facilitate polymerisation of the silicates[5] as is the presence of calcium and magnesium[6], all of which are found in high concentrations in the CSG water.

The silicon concentration in a typical CSG water treatment process at the micro-filter feed (process point |2| in Figure 1) is approximately 12 mg/L. After the RO process the silicon concentration in the RO concentrate/Brine (process point |3|) increases to 50-125 mg/L.[2]
Silica fouling is a problem in most industrial processes that incorporate a process which concentrates salts in the water. Industrial processes with silica fouling challenges include power generation, pulp milling, geothermal energy generation and Reverse Osmosis water treatment plants. The most common method for removing silicates to prevent fouling is lime softening involving the addition of slaked lime $\text{Ca(OH)}_2$ [7] [8]. A related and next most common method is precipitation/coagulation with multivalent metal hydroxides $\text{Fe(OH)}_3$, $\text{Al(OH)}_3$ [9], $\text{Mg(OH)}_2$ [10] [11] or metal salts [12]. Other methods such as electro coagulation [13], and silica adsorption, usually aluminium hydroxide adsorption [2] have also been used. These all produce a waste and are not always effective. For example, the precipitation based methods generate a metal-rich sludge while regenerating adsorbents generate an acidic or caustic waste stream.
1.2. Thesis Objective – Biological Silicon Removal

This project explores the potential for a bio-based process for silicon removal. To be effective, the biological alternative (diatoms) must meet two criteria: firstly be cost comparable to traditional processes such as slaked lime and secondly achieve a similar reduction in dissolved silica. Whilst a cost comparison was not undertaken, it has been assumed that if diatoms were grown in existing CSG holding ponds and extracted by existing micro-filters the biological process would be cost comparable to traditional processes.

To determine if the biological alternative can achieve a similar reduction in dissolved silica as traditional processes, the reduction rates of traditional processes must be understood. Three traditional processes were considered for comparison;

1. Activated Aluminium adsorption column using a GHD patented process [14]: This process has been found to reduce RO concentrate dissolved silicon from 75 - 120 mg/L to 25 – 50 mg/L (67% to 60% reduction).

2. Dowex 21 K XLT in an ion exchange column: An ion exchange case study [15] found a reduction of dissolved silicon from 20-16 mg/L to 6 – 14 mg/L, over 200 minutes (68% to 13% reduction).

3. Lime treatment: A lime treatment case study [8] found using 2,200 mg/L of lime (800 mg/L lime increased by 1,400 mg/L for 1,900 CaCO3 alkalinity) provided a reduction in dissolved Si from 62 mg/L of to 4 mg/L (95% reduction).

With consideration of these traditional methods it is assumed the biological process would be effective if it achieved an 80% reduction in dissolved silicon.

To achieve 80% reduction in dissolved silicon in a typical CSG water treatment process dissolved silicon (DSi) at process point |4| in Figure 1 must be 12 mg/L or less. To achieve 12 mg/L DSi at process point |4|, a DSi of 2 mg/L at process point |2| is required. If this is achieved the silica and hardness process step, between process points |3| and |4|, could be removed.
1.3. Thesis Organisation

The structure of this thesis is first a literature review of silica in water and diatoms, then objectives and scope, methods, results and finally the conclusion. The results section discusses the likelihood of a diatom based biological process achieving the target of 2 mg/L dissolved silicon at process point |2| and thus being an effective alternative.
2. LITERATURE REVIEW

A literature review was undertaken to understand the current research on the following aspects of dissolved silicon in water and diatom growth:

- The form(s) that silicon is likely to be in in CSG water;
- The biological process of diatom growth, as the growth and silicification of diatoms is intrinsically linked to their ability to remove dissolved silica; and
- The methods of measurement and identification to determine the dissolved silicon and silicon uptake into the diatoms to confirm silicon is being removed by the diatoms.

The treatment and use of the diatoms and silicon once removed from the CSG water was not investigated as it is outside the scope of this research. The following sections describe the current literature on each aspect.

2.1. Silicon in Water

Silicon is generally found in the following forms in water:

- Dissolved silicon: silicic acid;
- Colloidal silicon: suspension of amorphous silica (Figure 3) and metal silicates; and
- Biological silicon: amorphous crystalline quartz silica, \( \text{SiO}_2\cdot\text{nH}_2\text{O} \), diatom frustule.

Together these three forms are described as total silicon in this research.
There was expected to be a direct link between the reduction in dissolved silicon and creation of biological silicon. Colloidal silicon was not expected to change form during the experiments as the pH and salinity will remain relatively stable in the flasks and tubs.

However, the silica in the holding ponds (refer Figure 4, which is an extract from concurrent research into silica fouling [2], appeared to change between colloidal/particulate and dissolved silica. The total amount of silicon in the holding pond increased then decreased and proportion of dissolved and particulate (biogenic and colloidal) silicon changed, between the holding pond intake raw (brackish in Figure 4) water, and post micro filtration (RO feed in Figure 4). The reasons for this change were not investigated but could in part be due to the removal of colloidal silica by the micro filters and the reinjection of colloidal silica into the holding ponds. The effectiveness of a biological process relies on transforming dissolved silica to biological silicon, which should settle or be removed by the micro filter along with colloidal silica.
Figure 4: Changes in total silica from raw (brackish) intake water to brine (RO Reject). [2]
2.2. Biological Process of Diatom Growth

The organic process to propagate biota, usually involves one of the follow two methods [16]:

- A controlled process (closed photo bioreactor) with tight control of environmental conditions; or
- An open process with limited control of environmental conditions.

Open processes are less productive but also require less capital investment. This study focusses on the less capital intensive open process. To simulate as closely as possible an open process, propagation of the diatoms in the existing collection or brine ponds, the diatoms were grown in small open tubs in an outdoor environment as well as in the laboratory. This open environment faced the challenges of variable light, temperature and exposure to wind blown pathogens and algae competitors.

2.2.1. Diatoms

Whilst most marine plankton use the macronutrients/chemicals such as carbon, nitrogen and phosphate, silicon is an essential chemical only for specific biota, such as diatoms [17]: radiolaria, silicoflagellates, and siliceous sponges. These biota extract dissolved silicon from the water to build their cell walls (spicules, scales, solid plates, granules and frustules). The focus of this research was to investigate whether this biological dissolved silicon extraction process can be utilised to remove dissolved silicon from the coal seam gas (CSG) water.

Diatoms are the most common biota (there are approximately 100,000 diatom species) that uses dissolved silica to build its cells [18]. They provide more than 40% of the ocean’s primary production, and have extracted most of the silicon from the world’s oceans over the last fifty million years [19]. They are however, quite adaptable to varying proportions of silicon and other macronutrients and can thrive in high and low silicon environments characterized by high and low silicification (greater or less than 0.15 Si/C) with a range of 0.04 to 0.42 Si/C [20].
Diatom specific growth rates with a doubling time less than a day [21], can be described as high. Specific growth rate [22] is defined as \((\ln N_2 - \ln N_1) / (t_2-t_1)\), \(N_2\) and \(N_1\) are the cell densities at \(t_2\) and \(t_1\). Noting extremely high specific growth rates 4-8/day have been recorded in laboratory conditions where light, nutrients and temperature are optimized [21].

The CSIRO Australian National Algae Culture Collection method describes five reasonably well defined phases of algal growth; lag, exponential, declining, stationary and death. Exponential growth rates during the exponential phase are recommended by the CSIRO Australian National Algae Culture Collection methods and adopted by the UQ Agriculture and Food Science laboratory. As there are potential errors when using exponential biomass growth rates in light and nutrient limited conditions, exponential growth rates will primarily be used to demonstrate that biomass is increasing whilst dissolved silicon is reducing. Conclusions using growth rates are to be considered with regard to the potential error in the rates. Furthermore, the growth rates reported will be the average growth rates.

Diatoms have a wide distribution ranging from the Arctic to the Antarctic, but grow most prolifically near the equator. They are also found in a range of water salinities, from fresh to estuarine to open oceans to extremely saline environments. Diatoms thrive in the upper levels of open water, or in/near the bottom and some live in moist soils [23]. In summary, as a species they have evolved to populate most water environments on earth. Hence, diatoms are likely present in the CSG water and this investigation seeks to understand why diatoms appear to not be present in CSG water.

Diatoms belong to the class Bacillariophyceae [24] and their general characteristics are:

1. Size: 2 um to 2mm [18];
2. Cellular: Unicellular, often in colonies [18];
3. Vegetative reproduction by binary fission [24]. Following reduction in cell size after fission, the cell size is increased by auxospores; and
4. Their cell walls are amorphous quartz silica [25].

Due to the large range of species, extensive range of environments where they thrive and relative abundance, diatoms have been selected as the biological agent for this research.
2.2.2. Factors Influencing Diatom Growth

Diatoms growth rates are influenced by the following factors:

1. Water characteristics (salinity, pH) [16], [26], [27];
2. Light availability [28], [29];
3. Water temperature [30];
4. Bacteria and viruses, and competition from other algae [31];
5. Diatom growth characteristics (and silicon content) [32], [33];
6. Mixing [34];
7. Macronutrients such as carbon, phosphate and nitrogen [35], [36], [37], [38]; and
8. Micronutrients such as Boron and Iron [39].

Whilst some literature [32] suggests larger cell sizes grow faster than smaller cells this appears to be a result of the above factors so has not been included as a factor in itself.

To simulate the ponds environment the following factors were identified in the pond(s) and replicated as closely as possible in the investigation;

1. **Water characteristics (salinity, pH):** Diatoms grow in a range of water salinity and pH. Therefore the selection of a robust diatom(s) suited to the CSG water salinity and pH environment should negate the need to vary the water characteristics. Additionally there is a large volume of water to be treated each day (approximately 10 ML/day which is forecast to rise to 40 ML per day [40] at each water treatment plant) and dosing to bring about a meaningful change in salinity or pH is likely very expensive and would negate any potential benefits of the biological solution.

2. **Light availability:** The growth of diatoms is dependent upon light for photosynthesis, and as the water depth increases the available light decreases. Photosynthetically Active Radiation (PAR), the light wave length used for photosynthesis, can reduce to approximately 20% of surface PAR at a depth of 6m [41]. Diatom growth rates can be low (0.3/day) at irradiance 18 μmol/m².s and increase as irradiance increases up to 50 μmol/m².s, but there can be little change in growth rate as irradiance increases from 50 to 500 μmol/m².s [29] (1,700 μmol/m².s is equivalent to the World Meteorological Organisation definition of sunshine, 120 W/m [42]).
Diatoms maximum growth depth varies according the species, for example:

- *Coscinosira polychorda* maximum growth is at 1 to 2m below the surface [43, 44];
- *Delicata delicatula* maximum growth is at 1 to 5m below the surface; and
- *Adlafia bryophila* maximum growth is at 5 to 15m below the surface. [41]

There appears to be an optimum depth for the growth of each diatom which is a balance between light required for photosynthesis, nutrient availability and temperature. The optimum depth varies considerably between species. As the CSG pond depths are approximately 2.5m, diatoms will be selected that are adapted to growing in light at depths less than 2.5m.

The large water surface area available in the ponds, and the dry subtropical environment, 65 rainy days per year and average temperature 5 to 35 °C [42] will likely provide adequate light for the diatoms. The variability of light with changes in weather is a challenge that is inherent in open processes and no attempt will be made to control the available light. Rather the process will need to be sufficiently robust to accommodate variable light conditions. The robustness of the process will be tested in the outdoor trials in Brisbane which has on average 80 rainy days per year and average temperature 10 to 30 °C [42].

3. **Water Temperature:** Some diatom’s (*Cyclotella cryptica*, *Thalassiosira eccentrica*) growth rate increases with increasing temperature, however their cell volume decreases. Other diatoms such as (*Chaetoceros simplex*, *Thalassiosira weissflogii*) growth rate increases with water temperature [30] but has little growth rate increase above 16 °C. However, *Chaetoceros muelleri* which is accustomed to higher water temperatures has an optimum growth rate between 30 and 35 °C.
There appears to be a number of diatoms suited to a range of water temperatures. The water temperature in the CSG ponds is expected to be between 5 and 35 °C [45] and diatoms species will be selected for their adaptability to the prevailing water temperature. Their suitability for the likely temperature range in the CSG ponds will be tested to some extent by the outdoor trials in Brisbane which has on average a 5 °C higher minimum average temperature and 5 °C lower maximum average air temperature than the CSG fields.

4. **Competition from algae, bacteria or virus:** There are a number of pathogens which can adversely affect diatom growth [31, 46] and numerous algae which can compete with diatoms for available light and nutrients. As restricting growth of other algae is not easily managed (algae use the same principal C:N:P macro nutrients) diatoms which are suited to the existing pond and water characteristics will be selected, and their nutrient supply improved so they can out compete other algae. This approach has been successful in other studies [47], [48], [49]. To manage the potential pathogen risk a range of diatoms will be made available to remove silicon should one species be attached by a pathogen.

Accordingly, the above factors (water, light, temperature, carbon and competition) which are characteristics of the pond(s) and CSG water were not varied or controlled in this research as they are likely cost prohibitive to vary, and inherent challenges in open processes.

By excluding the above factors, the following four factors that influence diatom growth were investigated;

A. Diatom species;
B. Mixing;
C. Macronutrients; and
D. Micronutrients.
These four factors that influence growth are described further below.

A. Diatom Species:
Different diatom species thrive in different conditions and require small or large amounts of silicon to build their frustules. A range of different types of diatoms have been researched. However, most research has been undertaken in the northern hemisphere in and around the sea upwelling on the northern coasts of North America and Europe. Due to the location of this research most of the diatoms investigated thrive in cool temperate to cold polar regions, which are not similar to the dry subtropical environment of the CSG ponds. There is however research which shows diatoms thrive across a range of regions and the following are noted for their diversified characteristics and ability to thrive in the subtropics;

- *Chaetoceros muelleri* [50] (lipid production and appetite for dissolved silica).
- *Phaeodactylum tricornutum* (specific growth rate 1.5/day [51])
- *Amphora sp* (specific growth 0.15/day, benthic characteristics [52])
- *Aulacoseira sp* [53] (lower salinity adaptation, endemic to the Murray River);
- *Cyclotella striata* [53]. (heavily silicified Eastern Australia estuary diatoms); and
- *Thalassiosira weissflogii* [54] . (heavily silicified, specific growth rate 1.5/day [17]).

A range of diatoms which are likely to thrive in CSG water were selected and trialled.

B. Mixing: The mixing of water is important for diatom growth [47]. In the natural environment abundant diatom growth occurs where there are upwelling’s of nutrients, which allows the nutrient rich deeper water to mix with the dissolved carbon in the presence of sunlight to provide the necessary nutrients for photosynthesis. These areas of upwelling such as the west coast of North America, above the tropic of Capricorn on the west coast of South America and east coast of Australia and New Zealand provide more than 50% of the oceanic plankton growth [55]. This mixing, ensuring nutrients are available for the diatoms, was replicated in the laboratory though air bubblers, spinning discs or water pumps.
C. Macronutrients: Diatoms are primarily formed using carbon, nitrogen, silicon, sulphur, potassium and phosphorous (Macronutrients) [56], [57], [33], [58], [60]. For diatoms to form, these elements must be biologically available from the water, or introduced as carbon which is sometimes is introduced from the atmosphere. The following sections provide guidance on the optimum ratios of these macronutrients relative to each other. Whilst diatoms are adaptable to different ratios of macronutrients, their growth can be limited if one macronutrient is not available at the optimum ratio. To ensure the diatom growth is not hindered in the CSG water, the following literature study was undertaken to determine the optimum ratios and if necessary correct the deficiencies by the addition of commonly manufactured chemicals.

(1) C:N:P: Carbon availability is critical for diatoms growth as approximately 50% of diatom mass is carbon. It is generally accepted and confirmed by a number of different studies [61] the C:N:P ratio in diatoms should approximate the Redfield ratio C:N:P = 106:15:1. In addition to being present the carbon, nitrogen and phosphorus must be biologically available. Whilst some historical research [62] suggest CO₂ is the only source of carbon for diatoms, more recent research [36] suggest both CO₂ and HCO₃⁻ are directly available for diatoms. Assuming Redfield’s ratio is applicable for diatoms, and the diatoms can access the carbon from HCO₃⁻ [35] there appears to be abundant inorganic carbon available for diatom growth in the CSG water as shown below in Table 4. Nitrogen must also be available in the form of urea, ammonium, nitrites or nitrates [63] and phosphorus in the form of PO₄³⁻ [64].


(3) Sulphur: Sulphur is also a significant constituent of diatoms. The following Table 1 describes the ratios from sourced literature, noting that it references the frustule and not the complete diatom.
Table 1: Literature Sulphur ratios

<table>
<thead>
<tr>
<th>Reference:</th>
<th>Surface chemical composition of diatoms[60]</th>
<th>Silica and Carbonate ions requirements in freshwater diatoms[58]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Molar Ratio S: Si</strong></td>
<td>2.3:1 to 0.5:1</td>
<td>1.5:1 to 0.6:1</td>
</tr>
<tr>
<td><strong>Molar Ratio S: C</strong></td>
<td>1:70 to 1:100</td>
<td>X</td>
</tr>
<tr>
<td><strong>Molar Ratio S: K</strong></td>
<td>6:1 to 3:1</td>
<td>X</td>
</tr>
</tbody>
</table>

(4) Potassium: Whilst not found in the same proportion as sulphur and silicon, potassium is found in diatoms in similar ratios to phosphorous as shown in table 2 below.

Table 2: Literature potassium ratios

<table>
<thead>
<tr>
<th>Reference:</th>
<th>Effect of Phosphate on growth of diatoms [65]</th>
<th>Surface chemical composition of diatoms</th>
<th>Elemental composition of diatoms [66]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Molar Ratio K:Si</strong></td>
<td>1:7</td>
<td>1:3 to 1:6</td>
<td>1:11</td>
</tr>
<tr>
<td>(K, 0.05mM: Si, 0.35mM)</td>
<td>(based on Si=S)</td>
<td>(based on N=Si)</td>
<td></td>
</tr>
<tr>
<td><strong>Molar Ratio K:N</strong></td>
<td>1:11</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Molar Ratio K:S</strong></td>
<td>1:3 to 1:6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

D. Micronutrients: Micronutrients such as Iron are essential for phytoplankton growth, and required for photosynthetic and respiratory electron transport, nitrate and nitrite reduction, chlorophyll synthesis and other biological processes [67]. Iron seeding has been found to be particularly helpful to promote diatom growth although there appears to be a large range of optimum concentrations and molar ratios for Fe$^{3+}$ with other nutrients [37]. Deficiencies of Boron, Manganese and Magnesium have also been found to adversely affect diatom growth [66], [68], [69].

(1) Iron: The optimum Fe$^{3+}$ concentration ranges from 0.5 mg/L to 2 mg/L [67]. The Redfield ratio C:N:P = 106:16:1 has been expanded by many texts to include a ratio for Fe$^{3+}$. There is however a large variance in the recommended Fe$^{3+}$ ratio. The recommended ratio varies from C:N:P:Fe = 106:16:1: 0.0003 [37] to 0.00004 [70].
(2) Boron: Boron deficiency has also been found to limit cell growth. Boron is an essential element in diatom growth [68]. In one reference after 7 days, $4 \times 10^6$ diatom cells had grown in 0.5 mg/L of boron (0.003 B:Si) whilst only $4 \times 10^4$ had grown in 0.02 mg/L of boron [71].

(3) Magnesium Magnesium is used by diatoms and other plants as an element of the chlorophyll molecule, a carrier of phosphorus and enzyme activator. Table 3 below provides the range of magnesium to nitrogen and potassium weight ratios, which have been converted to molar ratios

Table 3: Literature review of Magnesium ratios

<table>
<thead>
<tr>
<th>Reference: Elemental composition of diatoms [66]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molar Ratio Mg: N 1:26</td>
</tr>
<tr>
<td>(15g N to 1g Mg)</td>
</tr>
<tr>
<td>Molar Ratio Mg: K 1:10</td>
</tr>
<tr>
<td>(3.5g K to 1g Mg)</td>
</tr>
</tbody>
</table>

(4) Manganese Manganese has been found to offset the detrimental effect of high copper concentrations (greater than 100 nM) [69]. It has also been found to offset a deficiency in Fe$^{3+}$ [72] when it is available in concentrations of 5 to 15 nMol. It appears to promote growth when provided in similar ratios to carbon: Fe$^{3+}$ ratio.

(5) Copper: Copper can both inhibit and promote diatom growth and has been found to be a cofactor of electron-transfer proteins and superoxide dismutases (enzymes that catalyze the dismutation of superoxide (O$_2^-$) into oxygen and hydrogen peroxide) [73]. There appears to be an interrelationship between Cu and Fe, where the absence of one is compensated by the provision of the other. High growth has occurred when the ratio of copper: silicon was 1:1,000 and the ratio of copper: Fe$^{3+}$ was 1:100.
2.3. Silicon and Diatom Quantification

The literature review and the methods selected to quantify the different forms of silicon in water, and volume/weight of diatoms are listed below.

2.3.1. Silicon

**Biological Silicon:** Biological silicon can be difficult to measure [74] as it is challenging to separately identify the colloidal silicon and biological silicon. To measure the biological silicon it is necessary to first classify biological silicon as the diatom frustule silicon, [25] which can be described as amorphous but crystalline quartz. The biological silicon (BSi) measurement has historically been based on soil based amorphous silica measurement techniques [75] but this has been found to contain errors when used for water based diatoms. The process to measure biological silicon developed by Ragueneaua et al [76] summarized in Figure 5, has been adopted by other thesis [77] and is proposed for this research. It comprises of a double wet-alkaline digestion where it is assumed all the BSi and part of the colloidal silicon have been converted into Si(OH)$_4$ by the first digestion and silicon and aluminium digestion ratio for colloidal silicon is unchanged for the second digestion. Following the analysis of Si$_1$ and Al$_1$ from the first digestion the filter sample is digested a second time, leading to the determination of the (Si:Al)$_2$ ratio of colloidal silica. The biological silicon concentration is calculated as $\text{BSi} = \text{Si}_1 - \text{Al}_1 \times (\text{Si}:\text{Al})_2$.

![Figure 5: Diatom silicon content method 1st leach, a second leach is required.](image-url)
**Dissolved Silicon**: A number of studies have measured dissolved silicon using the Molybdosilicate colorimetric method (HACH 8185), and this method was adopted for this research. However, this method is affected by the dissolved salts in the water [78] and the recommended matrix check was undertaken to minimise the influence of the salts on the results. In addition the dissolved silicon measured from Molybdosilicate colorimetric method was verified using ICP data.

### 2.3.2. Diatoms

Observation of diatoms can be challenging and many methods have been developed for the identification of lipids in diatoms through a red dye [79] which is not of interest for this research. The microscopic observation will follow the Australian National Algae Culture Collection Methods which involves a number of washes and centrifuges to remove the organic matter and leave the silicon frustule intact. Particularly it recommends Naphrax with a refractive index of 1.65 to 1.73 to assist visibility of the diatoms under a microscope.

The diatom growth rate can be measured by counting daily changes in cell numbers, weighing biomass, and/or changes in optical density, using both 610nm [80] and 750 nm [50]. To speed the process the 610 nm optical density was measured and correlated to total suspended solids, which approximates the biomass density. The 610nm wavelength was selected as it the wavelength closest to the brown colour of the diatom and green algae. The total suspended solids were assumed to be an accurate reflection of the biomass. The biological silicon measurement was used to determine the diatom proportion of the biomass.
3. RESEARCH DIRECTION, OBJECTIVES AND SCOPE

3.1. Research Direction

Based on the review of the literature, the research direction for this project is outlined as follows.

A. Diatom Selection: The selection of a diatom with a high silicon content (heavily silicified) and/or high growth is likely to be more effective at reducing dissolved silica content. In addition it is important to identify a number of different diatoms to reduce the risk of process failure should the pond water conditions change or pathogens attack the resident diatom.

The following diatoms were selected for their:
- high silicon content (*Chaetoceros muelleri*-CM),
- high growth (*Phaeodactylum tricornutum*-PT); and
- tolerance for brackish water (*Fistulifera saprophila*-FS).

<table>
<thead>
<tr>
<th><em>Chaetoceros muelleri</em> [81]</th>
<th><em>Phaeodactylum tricornutum</em> [82]</th>
<th><em>Fistulifera saprophila</em> [83]</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Image of Chaetoceros muelleri" /></td>
<td><img src="image2" alt="Image of Phaeodactylum tricornutum" /></td>
<td><img src="image3" alt="Image of Fistulifera saprophila" /></td>
</tr>
</tbody>
</table>

*Figure 6: Selected inoculant diatoms*

*Thalassiosira weissflogii* which is used as aquaculture feedstock, available commercially and also heavily silicified 0.2 Si/C [39] was considered but not selected as it is not readily available in Australia.
B. Mixing: Mixing is an important factor to provide nutrients close to carbon and light sources for photosynthesis. The mixing methods available are bubbling air through the CSG water, paddle wheel mixing, waterfall cascade, rotation mixing/pumping the CSG water. Of these available types air bubbling and rotation mixing/pumping have been adopted and trialled.

C. Macronutrients: C, N, P, K, S: There is considerable literature which describes the optimum amount of each nutrient, in absolute concentration. However as the silicon concentration in the CSG is relatively high, the absolute concentrations have been translated into relative ratios to the silicon concentration. These relative ratios will be used to estimate whether sufficient macro or micro nutrients are available.

The optimum amount of each macronutrient, calculated to match the available silicon and is shown in Table 4 below.

**Table 4: Macronutrient ratios**

<table>
<thead>
<tr>
<th></th>
<th>Ratio relative to Silicon</th>
<th>Optimum concentration based on Si ratio</th>
<th>Holding Pond concentration (field measurement)</th>
<th>4:1 Diluted Brine concentration (lab measurement)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silicon</td>
<td>NA</td>
<td>NA</td>
<td>12 mg/L</td>
<td>12 mg/L</td>
</tr>
<tr>
<td>Carbon</td>
<td>7:1</td>
<td>94 mg/L</td>
<td>230 mg/L</td>
<td>Not measured</td>
</tr>
<tr>
<td>(Bicarbonate)</td>
<td></td>
<td></td>
<td>1,900 mg/L</td>
<td>Not measured</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>1:1</td>
<td>12 mg/L</td>
<td>5 mg/L</td>
<td>2 mg/L</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.15:1</td>
<td>2 mg/L</td>
<td>16 mg/L</td>
<td>32 mg/L</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.07:1</td>
<td>1 mg/L</td>
<td>1 mg/L</td>
<td>0.5 mg/L</td>
</tr>
<tr>
<td>Sulphur</td>
<td>1:1</td>
<td>12 mg/L</td>
<td>&lt; 1 mg/L</td>
<td>5 mg/L</td>
</tr>
</tbody>
</table>

From the above table it appears the CSG water is deficient in nitrogen, phosphorous and sulphur for diatom propagation.
D. Micronutrients: The following table provides a summary of the researched micronutrient ratios relative to silicon.

**Table 5: Micronutrient rations**

<table>
<thead>
<tr>
<th></th>
<th>Ratio relative to Silicon</th>
<th>Optimum concentration based on Si ratio</th>
<th>Holding Pond concentration (field measurement)</th>
<th>4:1 Diluted Brine concentration (lab measurement)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silicon</td>
<td>NA</td>
<td>NA</td>
<td>12 mg/L</td>
<td>12 mg/L</td>
</tr>
<tr>
<td>Iron</td>
<td>0.000006:1</td>
<td>0.000075 mg/L</td>
<td>0.400 mg/L</td>
<td>0.040 mg/L</td>
</tr>
<tr>
<td>Boron</td>
<td>0.003:1</td>
<td>0.040 mg/L</td>
<td>3 mg/L</td>
<td>1 mg/L</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.070:1</td>
<td>1 mg/L</td>
<td>1 mg/L</td>
<td>0.5 mg/L</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.000006:1</td>
<td>0.000075 mg/L</td>
<td>0.020 mg/L</td>
<td>0.002 mg/L</td>
</tr>
<tr>
<td>Copper</td>
<td>0.001:1</td>
<td>0.012 mg/L</td>
<td>0.040 mg/L</td>
<td>0.020 mg/L</td>
</tr>
</tbody>
</table>

Based on the researched ratios it appears there are sufficient micronutrients available in the CSG water for diatom propagation.
3.2. Objectives and Scope

This research answers the following research questions through trials undertaken regarding each sub-question;

A. Can diatoms remove dissolved silicon from CSG water?
   i. Can diatoms grow in the CSG water?
   ii. Is there a reduction in dissolved silicon commensurate with diatom growth in CSG water?

B. At what rate could diatoms remove dissolved silicon from CSG water?
   i. What is the relative mass of diatoms grown compared with the reduction in mass of dissolved silicon?
   ii. What is the relative reduction in dissolved silicon in laboratory compared to external containers?

The treatment and use/disposal of the diatoms and colloidal silicon once removed from the CSG water has not been investigated and is outside the scope of this research. However, disposal should not pose an insurmountable challenge as diatoms have beneficial uses.

Diatom beneficial uses include lipids for biodiesel [16] and protein/carbohydrates for animal (cattle and aquaculture) feedstock [84], [85], both of which are required close to the CSG fields.
4. RESEARCH METHODS

4.1. Water Characteristics

This study’s trials have been undertaken in water sourced from a CSG extraction facility in Queensland, Australia. Both raw water from the holding pond (point |1| in Figure 1) and diluted RO brine concentrate (point |3| in Figure 1) were used as the base growth media. Diluted brine was used to reduce the volume of water transported from the gas fields, and the dilution percentage (4:1 dilution, 20%) was selected to replicate the holding pond sodium and silicon concentrations. Typical diluted brine and receiving pond characteristics are shown in Table 6.

Table 6: Typical characteristics of holding pond raw water and diluted brine

<table>
<thead>
<tr>
<th></th>
<th>Holding Pond (field measurement)</th>
<th>4:1 Diluted Brine (lab measurement)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDS</td>
<td>6,500 mg/L</td>
<td>Not measured</td>
</tr>
<tr>
<td>pH</td>
<td>9</td>
<td>9 - 9.5</td>
</tr>
<tr>
<td>Sodium</td>
<td>2,400 mg/L</td>
<td>2,700 mg/L</td>
</tr>
<tr>
<td>Calcium</td>
<td>9 mg/L</td>
<td>30 mg/L</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>1,900 mg/L</td>
<td>Not measured</td>
</tr>
<tr>
<td>Silicon</td>
<td>12 mg/L</td>
<td>12 mg/L</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>5 mg/L</td>
<td>2 mg/L</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>1 mg/L</td>
<td>0.5 mg/L</td>
</tr>
<tr>
<td>Sulphur</td>
<td>&lt; 1 mg/L</td>
<td>5 mg/L</td>
</tr>
</tbody>
</table>
4.2. Inoculum

Inoculant diatoms *Chaetoceros muelleri-CM*, and *Phaeodactylum tricornutum-PT* were obtained from the University of Queensland Agriculture and Food Science laboratory and scaled up in the chemical engineering laboratory using 2L flasks, controlled 12:12 lighting, heat and mixing. The initial growth medium was seawater with a f/2 and silicon additive. Then the diatoms were transferred into a 4:1 diluted brine. Both a rotating cylinder and air bubblers were used for mixing.

In addition CSG raw water (from holding ponds) was obtained and placed in 2L flasks with controlled 12:12 lighting, heat and mixing. Macronutrients, sulphur, nitrogen and phosphorus were added to promote diatom growth. The biomass was extracted and isolated in the Agriculture and Food Science laboratory. Elements of the isolated biomass which appeared to contain diatoms under the microscope were scaled up and DNA extracted. The extracted DNA was analysed by the Australian Genome Research Facility Ltd.

Approximately 5% by volume of inoculum was added to each trial as this was considered to be the maximum viable commercial amount of inoculum considering the cost of installing inoculum tanks beside the CSG ponds and is the mid range of the recommended diatom inoculum volume recommended by the CSIRO. The inoculum TSS ranged from 1,600 to 4,000 mg/L resulting in a TSS of 80 to 200 mg/L in the containers.
4.3. Diatom Growth and Dissolved Silicon Reduction

Diatoms were grown in 2 L flasks and 30-50 L tubs (refer Figure 7) in the laboratory and outdoors. Artificial lights, 12 hours on 12 hours off, were used for illumination in the laboratory. The water temperature was approximately 20°C. Dissolved silicon (DSi) removal from the holding pond water and the diluted brine was tested. Inorganic carbon inherent in the CSG water was the carbon source.

![Figure 7: 30-50L and 2L Containers (30cm ruler for scale)](image)

Batch growth trials were conducted with various diatom species, mixing regimes and nutrient supplementations (Table 7). Triplicate “A” trials tested for growth and productivity of native diatoms in the raw water. Trials “B”, “C” and “D” investigated the productivity of different inoculums. Duplicate “E” trials investigated productivity in outdoor conditions and trial “F” considered multiple inoculums. Each trial ran for between 14 and 28 days and continued whilst nutrients were added to initiate the exponential growth phase then ceased once the dissolved silicon was less than 1 mg/L.
Native diatoms were grown by placing the CSG raw water under lights and adding nutrients. *CM, PT and FS* diatom inoculants were scaled up from cultures provided by the Agriculture and Food Science laboratory.

Fluorescent artificial lights available in the laboratory were utilised. The 1,000 lux provided by these lights was much less than the 80,000 to 550,000 lux (CIE Publication 85, Table 2) expected during sunshine hours. The average number of sunshine hours (as defined by the Campbell Stokes machine used by the Australian Bureau of Meteorology) for the outdoor trial “F” was 8. The relative effectiveness of the laboratory lights compared to sunshine (the likely commercialised light source) was tested in trial “E”.

The light source was consistent for all experiments except the outdoor experiments. During the outdoor experiments a number of rainfall events occurred although mostly evening / afternoon storms. An average of 8 hours of sunshine per day existed during outdoor trial “F”.

Temperature was not a variable considered in this investigation and was controlled by the laboratory air conditioning consistently between 21 and 24°C for the indoor trials.

Mixing was provided by air bubblers, rotation mixers and water pumps (refer section 3.1 B and table 7). The growth rate appeared to be independent of the mixing type.

pH was monitored in some experiments and found to be relatively stable in the containers that grew diatoms (the CSG water is well buffered).

Evaporation losses were compensated by the addition of distilled water. Samples were taken by pipette or syringe and generally 20ml was extracted every second to third day for nutrient analysis. The nutrients were added by the addition of dissolved K2HPO4, MnSO4, KNO3, MgSO4 and Na2SO4.

The biomass growth, diatom growth rate and increase in TSS expressed in this section is the average growth / increase in TSS over the exponential stage.
<table>
<thead>
<tr>
<th>Trial</th>
<th>Diatom</th>
<th>Water</th>
<th>Vol. (L)</th>
<th>Mixing/Aeration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ax3.</td>
<td>Native</td>
<td>Raw Pond Water</td>
<td>2</td>
<td>bubbler</td>
</tr>
<tr>
<td>B.</td>
<td>Chaetoceros Muelleri (CM)</td>
<td>Diluted Brine</td>
<td>30</td>
<td>pump</td>
</tr>
<tr>
<td>C.</td>
<td>Phaeodactylum Tricornutum (PT)</td>
<td>Diluted Brine</td>
<td>30</td>
<td>pump</td>
</tr>
<tr>
<td>D.</td>
<td>Fistulifera Saprophila (FS)</td>
<td>Diluted Brine</td>
<td>2</td>
<td>magnetic mixer then bubbler</td>
</tr>
<tr>
<td>Ex2.</td>
<td>PT</td>
<td>Diluted Brine</td>
<td>30</td>
<td>pump</td>
</tr>
<tr>
<td>F.</td>
<td>PT &amp; CM</td>
<td>Diluted Brine</td>
<td>50</td>
<td>pump</td>
</tr>
</tbody>
</table>
4.4. Analytical Methods

The measurement procedures were:

**Dissolved silicon:** The dissolved silicon was measured in accordance with Molybdosilicate colorimetric method (HACH 8185), and confirmed by ICP measurement. All samples were filtered by a 0.45 \( \mu \)m filter before testing.

**Dissolved nitrogen and phosphate:** A Lachat QuikChem8500 Flow Injection Analyzer was employed to determine the soluble available nitrogen (ammonium/ammonia, nitrite and nitrate concentrations) and soluble phosphorus (phosphate).

**Biological silicon:** The double wet-alkaline digestion process to measure biological silicon proposed by Ragueneau et al. [76] was adopted. This process was used in conjunction with ICP tests to measure the dissolved (DSi) biological (BSi) and colloidal silicon. Once the DSi and BSi was understood the Si mass balance was calculated and the removal of DSi as BSi confirmed.

**Silicon mass balance:** To confirm the dissolved silicon was removed by the diatoms, the mass of silicon in the biomass was compared with the mass of reduction in dissolved silicon. The comparison involved an overnight digestion of a biomass sample to ensure all biomass and particulate silicon was converted to dissolved silicon to allow measurement of the biomass silicon. The total biomass was determined by weighing the biomass captured on a 38 \( \mu \)m sieve and estimating the weight of biomass in the fluid that passed through the 38 \( \mu \)m sieve using a calibrated optical density measurement. The biomass retained on the sieve was then corrected for moisture content. The total silicon removed was calculated by multiplying the silicon proportion by the biomass weight. This was then compared to the reduction in dissolved silicon.

**Microscopic diatom observation:** The microscopic observation followed the Australian National Algae Culture Collection – Methods which involves a number of washes and centrifuges to remove organic matter and leave the silicon frustule intact.
Native diatom identification: Native diatoms were identified using the CSIRO microalgal isolation techniques of serial dilution and streak plating. Each sample was prepared by isolating cells using micropipettes within the cleaned vacuum chamber which were then placed in 96 well plates and streak agar plates, sealed and propagated in f/2 mixture in the Agriculture and Food Science laboratory controlled propagation room. Streak plating was found not to be effective but serial dilution was found to grow diatoms (as observed through a microscope) in some wells. The isolated diatoms were again propagated in the controlled Agriculture and Food Science culture rooms in f/2 medium in 100 mL flasks at 25 ± 1 °C, 12 h/12 h light/dark photoperiod at a light intensity of approximately 9,000 lux and constant bubbling condition until sufficient diatoms were available for their DNA to be extracted.

The DNA extraction was undertaken using a phenol: chloroform method. After extraction, genomic DNA within the 18S RNA region was amplified on a PCR machine by using the following primers: Forward 5’-GCGGTAATTCCAGCTCCAATAGC-3’ and Reverse 5’-GACCATACTCCCCGGAACC-3’.

PCR templates were then purified by using a Wizard SV Gel PCR Clean-Up System (Promega). For sequencing preparation, 5 μL of a 25 ng μL-1 PCR product were combined with 1 μL of a 10 μM solution of each of the above primers. The reaction was topped up to 12 μL with Millipore filtered water in a 1.5 mL tube and sent to Australian Genome Research Facility (AGRF) at The University of Queensland for sequencing analysis. The DNA protocol used was “Diversity Profiling – gDNA (GS FLX) – Roche GS FLX 454”
5. RESULTS and DISCUSSION

5.1. Diatom Growth and Dissolved Silicon Reduction

A number of trials ("A" to "F") listed in Table 8 were undertaken to establish if diatoms can grow in CSG water, could remove dissolved silicon from CSG water and the rate at which dissolved silicon could be removed. Examples of the diatoms that grew are shown in Figure 8 below.

![Figure 8: Biomass images from trials “C” and “A”: LHS PT, RHS native diatoms (40x)](image)

The average diatom growth rates in CSG water are shown in Table 8 and similar growth rates to the references below in other mediums were achieved.

- Diatoms can be expected to grow at 20g/m².day in f/2 medium [86]. This approximates to 3 mg/L.day silicon take up in the diatom (if diatoms are 15% by weight silicon and they grow to 1m depth), and 10 mg/L.day silicon take up if they only grow to 330mm depth.
- Dissolved silicon can be reduced from 18 mg/L to 2 mg/L in 11 days using Chaetoceros muelleri in f/2 medium [87] (1.5 mg/L.day DSi reduction rate)
The concentration of dissolved silicon (DSi) reduced concurrent with biomass/diatom growth in all trials. Figure 9 shows the estimated maximum rates of DSi reduction in each of the trials. The "A" trials demonstrated native diatoms would grow in the raw CSG water provided there was sufficient N, S and P biologically available. It was observed DSi reduction occurred for the Chaetoceros muelleri (CM) diatoms once a substantive biomass was established (trial “B”).

*Phaeodactylum tricornutum* (PT) diatoms provided a high rate of DSi reduction once sufficient macronutrients, N and P, were biologically available (trial “C”). *Fistulifera saprophila* (FS) diatoms in trial “D” provided an initial DSi reduction and then growth stalled until the pH was lowered.

There was no measurable difference in the DSi reduction rate between the indoor and outdoor trials (trials “C” and “E”), but multiple inoculums (PT and CM) did provide a greater DSi reduction rate in both mg/L.day and mg/m².day (trial “F”). It is expected the DSi reduction rate, expressed in mg/m².day will increase with water depth, as diatom growth will occur until approximately 2m depth [88] [44] [89] [90] and trials have only been conducted to 300mm.

Literature [29] suggests relatively constant diatom growth rate above 50 µmol/m².s (3,000 Lux) and light is known to be greater than 3,000 lux at 2m depth on a sunny day. Literature [43] [88] has also found diatom growth remains relatively constant up to 2m below the surface. This research suggests it is possible the DSi reduction rate, expressed in mg/m².day will increase with water depth up to approximately 2m in depth, depending on turbidity. This is likely as the growth per unit volume is expected to remain relatively constant but the total volume per unit area will increase with depth. It is expected that at greater than 2m depth the diatom growth expressed in mg/m².day will increase only slightly or remain constant as below this depth lack of light will restrict growth and growth per unit volume below 2m is expected to reduce significantly.
### Table 8: Batch growth trial results

<table>
<thead>
<tr>
<th>Trial</th>
<th>Diatom</th>
<th>DSI reduction rate</th>
<th>Biomass growth</th>
<th>Limiting factors</th>
<th>Container size</th>
</tr>
</thead>
<tbody>
<tr>
<td>“A”x3</td>
<td>Native</td>
<td>1.0</td>
<td>50</td>
<td>S: N: P</td>
<td>2L</td>
</tr>
<tr>
<td>“B”</td>
<td>Chaetoceros muelleri (CM)</td>
<td>1.0</td>
<td>120</td>
<td>10</td>
<td>Algae, N:P</td>
</tr>
<tr>
<td>“C”</td>
<td>Phaeodactylum tricornutum (PT)</td>
<td>1.5</td>
<td>220</td>
<td>140</td>
<td>N: P</td>
</tr>
<tr>
<td>“D”</td>
<td>Fistulifera saprophila (FS)</td>
<td>0.5</td>
<td>-</td>
<td>10</td>
<td>Turbidity, N:P</td>
</tr>
<tr>
<td>“E”x2</td>
<td>PT</td>
<td>1.2</td>
<td>210</td>
<td>-</td>
<td>N: P</td>
</tr>
<tr>
<td>“F”</td>
<td>PT &amp; CM</td>
<td>3.0</td>
<td>800</td>
<td>160</td>
<td>N: P</td>
</tr>
</tbody>
</table>

The biomass growth, diatom growth rate and increase in TSS expressed in this section is the average growth / increase in TSS over the exponential stage.

![Figure 9: Rate of dissolved silicon (DSi) reduction](image)

In most cases there appeared to be an activation period before diatom growth was observable. Trial “E” did not have an activation period, as it was inoculated directly from trial “C”, and commenced within 7 days of completion of trial “C”.
5.2. Biological Silicon

It was confirmed the reduction in dissolved silicon was primarily due to diatoms and not creation of colloidal silica by DNA analysis and silicon mass balance.

5.2.1. DNA Analysis:

A DNA analysis of the “A” trials (native diatoms) found the biomass was 85% Chlorella sp (Chlorophyta, green algae) and 15% Bacillariophyceae, diatoms (by number). As silicon is not evident in Chlorella algae and double wet alkaline digestion tests on trials “E” and “F” found biological silicon accounted for approximately 90% of the silicon removed (refer Table 9), it is reasonable to assume the reduction in dissolved silicon was primarily due to diatom growth. (refer to Table 8 for a description of the trials)

Table 9: Double Wet Alkaline Digestion

<table>
<thead>
<tr>
<th>Trial</th>
<th>Digest 1 (mg/L)</th>
<th>Digest 2 (mg/L)</th>
<th>BSi/Total Si</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Si (1)</td>
<td>Al (1)</td>
<td>Si (2)</td>
</tr>
<tr>
<td>Trial “E”</td>
<td>352</td>
<td>0.15</td>
<td>174</td>
</tr>
<tr>
<td>Trial “F”</td>
<td>87</td>
<td>3.8</td>
<td>5.2</td>
</tr>
</tbody>
</table>

5.2.2. Silicon mass balance:

To confirm the dissolved silicon was removed by the diatoms, the mass of silicon in the biomass was compared with the mass of reduction in dissolved silicon. The average biomass silicon content was 3% and the dry biomass retained on the sieve was approximately 25% of the wet weight. The total silicon removed, calculated by multiplying the silicon proportion by the biomass weight, was then compared to the reduction in dissolved silicon. This comparison of biomass silicon to silicon removed is presented Table 10 below.
Table 10: Silicon mass balance check (0.17 m² tubs)

<table>
<thead>
<tr>
<th>Trial</th>
<th>No days</th>
<th>Biomass weight (g)</th>
<th>Si removed 3% of Biomass (mg)</th>
<th>Si reduction rate 3% of Biomass (mg/m².d)</th>
<th>DSI density day 0 (mg/L)</th>
<th>Tub vol (L)</th>
<th>DSI Avail. (mg)</th>
<th>Si reduction rate DSI reduction (mg/m².d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>“E”</td>
<td>8</td>
<td>31</td>
<td>900</td>
<td>660</td>
<td>10</td>
<td>30</td>
<td>300</td>
<td>210</td>
</tr>
<tr>
<td>“F”</td>
<td>6</td>
<td>58</td>
<td>1,700</td>
<td>1,670</td>
<td>16</td>
<td>50</td>
<td>800</td>
<td>800</td>
</tr>
</tbody>
</table>

Total Biomass Produced: The total biomass grown in the tubs was estimated by measuring the mass of solids retained on the 38 µm sieve and estimating the biomass in the liquid that passed through the sieves. The wet biomass was converted to a dry biomass and the Total Suspended Solids (TSS) of the liquid passing through the sieve was estimated using a calibrated TSS optical density curve.

From 45 litres of CSG water and biomass, 197 g of wet biomass was retained on the 38 µm sieve. The dry mass retained on the sieve was estimated to be 45 g. This estimation was made by pro-rating the wet mass by the wet/dry ratio of 23% which was determined by drying a portion of the biomass under lights for two days and measuring its dry weight.

The optical density of the first 35 litres of water passing the 38 µm sieve was found to be 0.047 to 0.067. This approximates to 150 mg/L which is 5.2 g of biomass in the first 35 litres. The last 10 litres of liquid passing through the sieve had a higher TSS and an average optical density of 0.6 which approximates to 750 mg/L, 7.5 g of biomass.

This total dry biomass produced in 9 days was estimated to be 58 g (45g + 5.2g + 7.5g).
**Biomass Silicon Content:** To determine the silicon content, 53 mg of the biomass solids were diluted in 8 ml of 0.2M NaOH, heated to 100°C then filtered and centrifuged. The filtrate was tested and found to contain 198 mg/L of Silicon (1.6 mg of Si). The percentage Si was calculated to be 1.6 mg Si/53 mg of biomass, which is 3%.

**Silicon reduction calculated from proportion of silicon in biomass:** Assuming 3% of biomass was silicon, the total silicon removed by the biomass was 1,700 mg. (refer Figure 10)

![Figure 10: Silicon reduction calculated from proportion of silicon in biomass](image)

**Silicon Reduction calculated from reduction in dissolved silicon:** The silicon concentration of the container at day 10 was 16 mg/L and 0 mg/L at day 16. Resulting in 800 mg of silicon removed over the 6 days (16 mg/L in 50 L) as shown in Figure 11.
Figure 11: Silicon Reduction calculated from reduction in dissolved silicon

The results suggest 2 to 3 times more silicon was removed by the biomass (diatoms) than was available in a dissolved form. The estimation of silicon accumulated in the biomass was likely too high due to overestimation of the silicon in the biomass and silicon in inoculant biomass at day 0.

The measurement of silicon in the biomass is not accurate. Hence a relatively small (in measurement terms) change in portion of silicon (3% to 1-1.5%) will correlate the calculation of silicon removed using the reduction in dissolved silicon with the calculation using the increase in biomass and proportion of silicon in the biomass.

In addition it was observed that diatom growth occurred without any change in dissolved silicon for some time before the dissolved silicon reduction measured above. It is possible this was due a conversion of particulate silica in the inoculant biomass to dissolved silicon as the diatoms removed dissolved silicon. Whilst not conclusive these results support the DNA and double wet digestion results, that it is reasonable to assume the reduction in dissolved silicon was primarily due to diatom growth.

Diatom proportion of biomass: Assuming the Redfield-Brzezinski ratio is representative for these diatoms, a biomass with 3% silicon provides that diatoms are approximately 20% of the biomass.
5.3. Rate Limiting Nutrients

The absence of biologically available nitrogen, sulphur and phosphorus inhibited or prevented diatom growth. The concentration of available nitrogen required to initiate diatom growth appeared to be at least that of silicon.

The total phosphorus available before nutrients were added appeared to be adequate to commence diatom growth (15-20 µM P compared to 300-400 µM Si). However, a check of phosphorus $\text{PO}_4^-$ found biologically available phosphorus was 1-10 µM which was insufficient to sustain and initiate diatom growth. Although some diatom growth did occur at 20 µM phosphorus concentration, growth was limited and a minimum 100 µM sulphur and phosphorus was required before diatom growth was sustained.

Whilst literature suggests sulphur [60] should be available at the same ratio as silicon, the trials found provided sulphur is available at a 50% the concentration of silicon diatoms will grow.
Native diatoms: The correlation between the growth of native diatoms and dissolved silicon reduction is shown in Figure 12. Growth was stimulated first by the addition of sulphur and then by the addition of nitrate; the addition of phosphate had no effect on growth. The reduction in TSS at day 8 was due to the testing method which did not activate the settled diatoms. The final TSS measurement included vigorous mixing to ensure the settled diatoms were recorded by the optical density (OD) measurement which then converted to (TSS) by a calibrated OD-TSS curve.

Figure 12: Trial “A.2”, Reduction of silica concurrent with growth of native diatoms.
Inoculated diatoms: The correlation between growth and dissolved silicon removal was also observed in the inoculated trials. Growth of *Phaeodactylum Tricornutum* (PT) was stimulated by the addition of phosphate and nitrogen (Figure 13).

*Figure 13: Trial "C", Reduction of silicon concurrent with growth of PT diatoms*
Mixed inoculum: The maximum growth rate occurred with a mixture of diatoms and container depth of 300mm as shown in Figure 14.

Figure 14: Trial “F”, Reduction of silica concurrent with growth of PT and CM diatoms
5.4. Summary

The absence of biologically available nitrogen, sulphur and phosphorus inhibited or prevented diatom growth. The following table compares the literature suggested concentrations (refer section 2.2.2 C) in mg/L of available nitrogen (1N:1Si molar ratio), sulphur (1N:1S molar ratio), and phosphorous (1N:0.07P molar ratio).

Table 11: Minimum nutrients for diatom growth

<table>
<thead>
<tr>
<th>Trial</th>
<th>Si (mg/L)</th>
<th>Concentration for growth literature molar ratio (mg/L)</th>
<th>Concentration prior to growth (mg/L)</th>
<th>Concentration at start of growth (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N S P</td>
<td>N S P</td>
<td>N S P</td>
</tr>
<tr>
<td>A’x3.</td>
<td>5.5</td>
<td>2.8 6.3 0.4</td>
<td>0.7 6.3 0.8</td>
<td>5 20 10</td>
</tr>
<tr>
<td>B’</td>
<td>15</td>
<td>7.5 17 1.2</td>
<td>2.7 43 1</td>
<td>13 74 18</td>
</tr>
<tr>
<td>C’</td>
<td>14</td>
<td>7 16 1.1</td>
<td>2.6 17 0.6</td>
<td>14 11 10</td>
</tr>
<tr>
<td>D’</td>
<td>3</td>
<td>1.5 3.3 0.2</td>
<td>1 62 0.5</td>
<td>21 44 3.9</td>
</tr>
<tr>
<td>E’x2.</td>
<td>11</td>
<td>5.3 - 0.8</td>
<td>0.9 - 0.8</td>
<td>0.9 - 0.8</td>
</tr>
<tr>
<td>F’</td>
<td>14</td>
<td>7.1 16 1.1</td>
<td>2.7 4 1.9</td>
<td>18 3 3.5</td>
</tr>
</tbody>
</table>

In all cases (except trial “E”) as highlighted in bold in Table 11 the absence of biologically available nitrogen appeared to be limiting growth, as whenever biologically available nitrogen exceeded the literature molar ration to the available silicon, diatom growth started. The exception to this observation is that for trial “E” the absence of biologically available nitrogen did not appear to limit growth. As this trial was undertaken primarily to compare indoor to outdoor growth, the reason for the exception was not investigated.

The diatom growth rate varied from 0.8 to 5.5 g/m².d with a specific growth rate: 0.6 to 2.5 g/g.d. Noting the growth rate is up to 11 g/m².d if biomass (solids) and not the dissolved silicon reduction is used as the basis for the calculation, refer Table 10. Diatom growth led to the removal of dissolved silicon (Si) at a rate of 120-800 mg Si/m².d (or 1.2-8 kg Si/ha.d). The net biomass silicon content was 3 wt%. The lower growth rate occurred when trialling a single diatom species in 150mm deep containers, while the higher growth rate was achieved with multiple species in 300mm deep containers. It is expected diatom growth rate per unit area will increase with deeper containers until available light constrains growth.
For the biological process to be effective in CSG water (i.e. to achieve 80% reduction in dissolved silicon) the dissolved silicon concentration must be 2 mg/L at process point |2| in Figure 1. To achieve 2 mg/L at process point |2| for a typical 10 ML/day RO plant, 10 mg/L or 100 kg/day of silicon must be removed. If the mid range (500 mg Si/m².d) silicon reduction rate achieved in the lab can be achieved on site, a 20 Ha pond would be required.

Consequently existing CSG holding ponds could be utilized to grow the diatoms, but may need some augmentation and/or the diatom selection or nutrients would need to be further optimised.

Algae and diatom (biomass) harvesting can be undertaken using micro-screens, centrifugation, flocculation or a combination. As the CSG plants currently clean the CSG water with micro-screens / micro-filters prior to it entering the Reverse Osmosis membranes, it is proposed to use micro-screens /micro-filters to remove the biomass. In addition pond sludge removal could also remove benthic diatoms. It is expected the biomass removal would be continual.
6. CONCLUSION

Diatom growth can remove dissolved silicon from coal seam gas groundwater. Varying the diatom species and providing sufficient nutrients, notably nitrogen and phosphorus were found to promote growth both of the natural and introduced diatom species. The most successful species were found to be Phaeodactylum tricornutum and Bacillariaceae family although a combination of species was found to achieve higher silicon removal rates of 800 mg Si/m².d (or 8 kg Si/ha.d) for a 300mm deep tub.

For a biological agent to be effective removing dissolved silica it is likely the removal must occur without substantial capital investment, i.e. without additional ponds or micro filters. To utilise the existing ponds the growth rates and/or silicon content of the diatoms would need to achieve the higher laboratory rates. Existing micro filters are likely adequate provided sludge removal is effective. An additional pond/tank containing a pure inoculant would be required to manage the risk of an environmental, viral or bacterial event destroying the diatoms and interrupting the biological process whilst diatoms regrow.

Improved silica removal rates are possible through the use of deeper tubs (as the current tubs are only 300mm deep), providing suitable conditions for the higher silicon content diatoms such Chaetoceros muelleri (likely a host to increase turbidity), and providing a greater volume of inoculant (>5%).

The limitations of this thesis include: not investigating the effects of varying light and inoculum volume; and data from exponential growth may not be valid for simulating long term continuous systems. Still, it was found that diatom growth can remove dissolved silicon from coal seam gas groundwater. Although light and inoculum volume likely have a profound impact upon diatom growth rates it was found for this investigation that varying the diatom species and providing sufficient nutrients is likely to provide the basis for an effective biological alternative to conventional silicon removal processes.
LIST OF REFERENCES:


45. Sunwater. (2012), Nathan Dam EIS: Ecology of Fish Chapter 13 - Appendix 13A.


56. Brivaela Moriceau, Madeleine Goutx, Catherine Guigue, Cindy Lee, Robert Armstrong, Marie Duflos, Christian Tamburini, Bruno Charriere and Olivier


87. F.G Naghdi (2014) *Silicon reduction rate*; P. Rodman. 2014: Univeristy of Queensland Ag and Food Science Department


Appendix A. Laboratory Results
## Biological silicon removal from coal seam gas water

### Appendix A.

| Date   | Day | DSiO₂ | Dsi | Opt Den 610 nm | TSS | N as NOx | P as PO₄ | Al | B  | Cu  | Fe  | Mg  | Ca  | K  | P  | S  | Si | Comments / Nutrient addition |
|--------|-----|-------|-----|----------------|-----|----------|----------|----|----|-----|-----|-----|-----|----|----|----|-----------------------------|
| 12-Jun | 0   | 8     | x   | x             | x   | x        | 0.02     | 2.0| 0.00| 0.01| 5.9 | 7.1 | 7.9 | 0.7| 2.6| 8 | +56 mg/L KNO₃               |
| 17-Jun | 5   | 13    | 7   | x             | x   | x        | 0.02     | 1.7| 0.01| 0.01| 5.9 | 8.7 | 28.6| 0.3| 2.6| 4 |                                               |
| 18-Jun | 6   | 13    | 7   | 0.18          | 300 | x        | 0.08     | 1.7| 0.01| 0.01| 25.0| 8.7 | 28.6| 0.3| 25.0| 4 | +550 mg/L MgSO₄              |
| 20-Jun | 8   | 8     | 4   | 0.13          | 250 | x        | 0.02     | 2.2| 0.00| 0.01| 5.2 | 9.0 | 9.5 | 0.4| 1.4| 10                             |
| 23-Jun | 11  | 3     | 2   | 0.16          | 300 | x        | 0.02     | 2.2| 0.00| 0.01| 5.2 | 9.2 | 9.6 | 0.3| 1.5| 2 |                                               |

| Date   | Day | DSiO₂ | Dsi | Opt Den 610 nm | TSS | N as NOx | P as PO₄ | Al | B  | Cu  | Fe  | Mg  | Ca  | K  | P  | S  | Si | Comments / Nutrient addition |
|--------|-----|-------|-----|----------------|-----|----------|----------|----|----|-----|-----|-----|-----|----|----|----|-----------------------------|
| 25-Aug | 0   | 19    | 10  | 0.01           | x   | 0.7      | 0.3      | 0.14| 0.9 | 0.01| 0.08| 2.3 | 4.6 | 8.5| 0.8| 0.3| 7 |                                               |
| 27-Aug | 1   | 16    | 8   | 0.01           | x   | 0.5      | 0.1      | 0.08| 0.8 | 0.01| 0.04| 2.2 | 4.6 | 7.8| 2.6| 0.3| 6 | pH 9.3                                               |
| 29-Aug | 3   | 13    | 7   | 0.01           | x   | 0.2      | 0.1      | 0.07| 0.7 | 0.02| 0.03| 2.3 | 2.0 | 7.2| 1.6| 21.3| 5 | +250 mg/L MnSO₄                |
| 1-Sep  | 5   | 12    | 6   | 0.04           | 150 | 0.1      | 0.0      | 0.03| 0.8 | 0.03| 0.03| 1.9 | 1.2 | 7.4| 0.5| 22.9| 4 |                                               |
| 3-Sep  | 7   | 11    | 6   | 0.02           | 50  | x        | x        | 0.05| 0.7 | 0.02| 0.03| 1.5 | 1.1 | 7.2| 0.5| 22.9| 3 | pH 9.2                                               |
| 6-Sep  | 10  | 10    | 5   | 0.01           | 50  | 0.2      | 0.4      | 0.06| 0.9 | 0.01| 0.05| 1.6 | 1.1 | 22.0| 10.0| 22.4| 2 | +50 mg/L K2HPO₄                |
| 9-Sep  | 13  | 11    | 6   | 0.01           | 50  | 5.0      | 2.0      | 0.07| 0.9 | 0.01| 0.03| 1.7 | 1.0 | 61.0| 11.0| 22.0| 2 | +50 mg/L KNO₃                 |
| 13-Sep | 17  | 0     | 0   | 0.19           | 350 | 4.0      | 2.0      | 0.07| 0.8 | 0.00| 0.03| 1.8 | 0.9 | 59.8| 9.5 | 20.5| 0 |                                               |

| Date   | Day | DSiO₂ | Dsi | Opt Den 610 nm | TSS | N as NOx | P as PO₄ | Al | B  | Cu  | Fe  | Mg  | Ca  | K  | P  | S  | Si | Comments / Nutrient addition |
|--------|-----|-------|-----|----------------|-----|----------|----------|----|----|-----|-----|-----|-----|----|----|----|-----------------------------|
| 1-Sep  | 1   | 16    | 8   | 0.01           | 0   | x        | x        | x   | x  | x   | x   | x   | x   | x  | x  | x  | x  | pH 8.9                                               |
| 3-Sep  | 3   | 17    | 9   | x             | x   | x        | x        | x   | x  | x   | x   | x   | x   | x  | x  | x  | x  | pH 9                                               |
| 6-Sep  | 6   | 14    | 7   | 0.00           | 0   | x        | x        | x   | x  | x   | x   | x   | x   | x  | x  | x  | x  | +50 mg/L MgSO₄.7H₂O               |
| 9-Sep  | 9   | 14    | 7   | 0.00           | 0   | x        | x        | x   | x  | x   | x   | x   | x   | x  | x  | x  | x  | +50 mg/L KNO₃                |
| 13-Sep | 13  | 10    | 5   | x             | x   | x        | x        | 0.06| 0.7 | 0.02| 0.04| 7.1 | 4.5 | 23.9| 0.0| 7.0| 5 |                                               |
| 17-Sep | 17  | 9     | 5   | 0.25           | 500 | x        | x        | 0.06| 0.7 | 0.01| 0.02| 7.9 | 4.9 | 36.1| 0.6| 3.4| 5 | +50mg/L K2HPO₄                   |
| 22-Sep | 22  | 1     | 0.5 | 0.03           | 0   | x        | x        | 0.03| 0.7 | 0.02| 0.03| 6.4 | 4.7 | 47.8| 7.5| 4.5| 0 | Diatoms had settled.              |
# Biological silicon removal from coal seam gas water

## Appendix A

### Trial "B", CM, 20% RO, 30L tub 1.5L innoc

<table>
<thead>
<tr>
<th>Date</th>
<th>Day</th>
<th>DSIO2</th>
<th>DSi</th>
<th>Opt Den 610 nm</th>
<th>TSS</th>
<th>N as NOx</th>
<th>P as PO4</th>
<th>Al</th>
<th>B</th>
<th>Cu</th>
<th>Fe</th>
<th>Mg</th>
<th>Ca</th>
<th>K</th>
<th>P</th>
<th>S</th>
<th>Si</th>
<th>Comments / Nutrient addition</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-Jul</td>
<td>27</td>
<td>14</td>
<td>0.02</td>
<td>0</td>
<td>2.5</td>
<td>0.00</td>
<td>0.06</td>
<td>1.4</td>
<td>0.04</td>
<td>0.08</td>
<td>67.7</td>
<td>23.9</td>
<td>29.0</td>
<td>0.8</td>
<td>64.2</td>
<td>14</td>
<td></td>
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</tr>
<tr>
<td>12-Jul</td>
<td>27</td>
<td>14</td>
<td>0.01</td>
<td>0</td>
<td>x</td>
<td>x</td>
<td>0.03</td>
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<td>27</td>
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<td>0</td>
<td>x</td>
<td>x</td>
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<td>0.02</td>
<td>0.08</td>
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<td>x</td>
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<td>0.01</td>
<td>0</td>
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<td>x</td>
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<td>13.4</td>
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<td>0</td>
<td>x</td>
<td>x</td>
<td>0.04</td>
<td>1.1</td>
<td>0.01</td>
<td>0.04</td>
<td>45.7</td>
<td>13.7</td>
<td>17.4</td>
<td>0.5</td>
<td>59.9</td>
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<td>x</td>
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## Biological silicon removal from coal seam gas water

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### Trial "D", FS, 20% RO, 2L flask, 0.5L innoc

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