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Stabilisation of microalgae: Iodine mobilisation under aerobic and anaerobic conditions

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HIGHLIGHTS

- First investigation into iodine mobilisation during algae stabilisation.
- Useful amounts of iodine are retained during aerobic and anaerobic stabilisation.
- I mobilisation is correlated with C emission, indicating I present as organoiodine.
- N compared to I mobilisation indicates N and I are housed separately in algae.

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ABSTRACT

Mobilisation of iodine during microalgae stabilisation was investigated, with the view of assessing the potential of stabilised microalgae as an iodine-rich fertiliser. An iodine-rich waste microalgae (0.35 ± 0.05 mg I g^{-1} VS_{added}) was stabilised under aerobic and anaerobic conditions. Iodine mobilisation was linearly correlated with carbon emission, indicating iodine was in the form of organoiodine. Comparison between iodine and nitrogen mobilisation relative to carbon emission indicated that these elements were, at least in part, housed separately within the cells. After stabilisation, there were 0.22 ± 0.05 and 0.19 ± 0.01 mg g^{-1} VS_{added} iodine remaining in the solid in the aerobic and anaerobic processed material respectively, meaning $38 \pm 5.0\%$ (aerobic) and $50 \pm 8.6\%$ (anaerobic) of the iodine were mobilised, and consequently lost from the material. The iodine content of the stabilised material is comparable to the iodine content of some seaweed fertilisers, and potentially satisfies an efficient I-fertilisation dose.

1. Introduction

Iodine deficiency is a worldwide health concern. In 1980, about 60% of the world's population were iodine deficient (Zimmermann et al., 2008); iodized salt has since been supplemented into daily diets but still 30% of the world's population suffered iodine deficiency in 2007 (de Benoist et al., 2008). Additional strategies for iodine supplementation have been considered (Smoleń et al., 2014b). For example, increasing the iodine content of eatable plants was thought to be a safer and more efficient option than industrial iodized salt, as 75–85% of the iodine in human body comes from vegetable food under natural conditions (Weng et al., 2008). Increasing iodine in plants – iodine biofortification – is facilitated by using iodine-rich fertilisers.

Using iodine rich fertilisers can lead to some fruit and vegetables having an iodine content of several mg kg^{-1} (dry weight), e.g. carrot: 1 mg kg^{-1}, tomato: 6 mg kg^{-1}, soybean: 3.5 mg kg^{-1} (Dai et al., 2004; Weng et al., 2014); leaf vegetables tend to have relatively higher iodine content, e.g. spinach: 65 mg kg^{-1}, celery: 50 mg kg^{-1}, Chinese cabbage: 80 mg kg^{-1} (Nkemka and Murto, 2010; Singh and Ajay, 2011). Researchers have shown a linear relationship between iodine uptake rate and exogenous iodine (I^-, IO_3^-) level (0–10 mg L^{-1}) for cabbage and celery (Hong et al., 2009). Foliar fertilisation with KIO_3 at 1 mg I L^{-1} can result in lettuce leaves having an iodine content of 800 mg kg^{-1} (Smoleń et al., 2014a), which is comparable to some high iodine content seaweeds (Kupper et al., 2008).

Algae have long been recognised as an important iodine accumulator in natural environments (Kupper et al., 2008). With the increasing interest in iodine biofortification, algae of high iodine content have been considered as a fertiliser. For example, ground dry kelp (0.2–0.3% iodine) was found to be an effective fertiliser

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for increasing iodine content in soil and improving iodine uptake by plants (Weng et al., 2008). The study showed kelp fertilisation of 40 mg kg^{-1} (iodine to soil) could provide a residual iodine content of 25 mg kg^{-1} in soil (free iodine) after 90 days plantation. Meanwhile, during the plantation, the iodine content of eatable plants was elevated to significant levels, e.g. cucumber: 6 mg kg^{-1}, aubergine: 10 mg kg^{-1}, radish: 8 mg kg^{-1}. Weng et al's. (2008) study also found kelp as an iodine fertiliser can slow down iodine release in soil compared with potassium iodide (KI). Every year there are large amounts of waste algae generated from aquatic systems in many places around the world (Han et al., 2014). Algal fertiliser for iodine biofortification could represent a new opportunity for utilisation of waste algae with high iodine content. However, before applying to land, organic waste must be stabilised to avoid adverse environmental consequence (Polprasert, 1996). Stability refers to the rate or degree of organic matter decomposition, and can be expressed as a function of microbiological activity (Wu et al., 2000). It has been concluded that stabilisation increases the agricultural value of the material as a consequence of organic matter humification, reduces pathogens, and destroys some toxic compounds (Sanchez-Monedero et al., 2004). But there are currently no reports concerning iodine mobilisation during algae stabilisation.

In this study, iodine-rich waste microalgae harvested from a groundwater holding pond was stabilised under both aerobic and anaerobic conditions. The organic carbon degradation and nutrient and iodine mobilisation were monitored. This is the first work considering iodine mobilisation during organic waste stabilisation. The outcomes are significant in the context of utilising the final products as fertiliser for iodine biofortification.

2. Methods

In this work, 'waste algae' refers to algae harvested from a laboratory algal growth system (the laboratory growth system represents a groundwater holding pond, with iodine rich groundwater used as the growth medium). The waste algae was stabilised in (i) an aerobic environment, using activated sludge from a wastewater treatment plant as the inoculum for stabilisation, and (ii) an anaerobic environment, using digester sludge from a wastewater treatment plant as the inoculum for stabilisation.

2.1. Waste algae and inoculum for stabilisation

Waste microalgae was collected from a lab scale production system that used iodine-rich groundwater (2.0 mg iodide L^{-1}) as the water source. The lab scale algal production system consisted of a 5 L large glass beaker illuminated with florescent light of 80 μmol m^{-2} s^{-1} at 25 °C using the groundwater as medium. After 4 months cultivation, the microalgae biofilm in the beaker was harvested and drained on a 500 μm pore size mesh plate. Then the mesh plate with algae was placed in a 4 °C fridge overnight for further drainage.

To decompose the waste microalgae, wastewater treatment sludge was used as inoculum. For aerobic digestion, activated sludge was obtained from the aerobic zone at Luggage Point wastewater treatment plant (LPWWTP), Brisbane, Queensland, Australia. For anaerobic digestion, digester sludge was obtained from the same treatment plant.

2.2. Waste algae stabilisation

Carbon emission was used as the measure of stabilisation. Stability refers to the rate or degree of organic matter decomposition, and can be expressed as a function of microbiological activity (Wu et al., 2000), which in this case was determined by CO_2 and CH_4 production.

The experimental set-ups for waste algae stabilisation are shown in Fig. 1. For aerobic digestion, 30 g wet algae, 15 g inoculum and 115 g distilled water were mixed in a 240 ml glass reactor. A magnetic stirrer was used for mixing. As shown in Fig. 1, input air flowed through two 240 ml glass bottles, the first containing saturated NaOH solution (200 ml), the second distilled water (200 ml), to ensure a CO_2 free and water saturated air feed. After each reactor, there was another 240 ml glass bottle containing 100 ml NaOH solution (2 mol L^{-1}) for trapping the CO_2 from exhaust gas. The four bottles were connected by tygon tubing in series for one way ventilation by compressed air. The aerobic stabilisation trial was repeated 4 times. The negative control, which was carried out in duplicate, contained additional water in place of the algae. Detailed set-up conditions are shown in Table 1.

For anaerobic digestion, 30 g wet algae, 70 g inoculum and 60 g distilled water were mixed in 240 ml glass bottles, which were then sealed with butyl rubber stoppers. Before being sealed, the headspace was flushed with nitrogen gas. The anaerobic stabilisation trial was also repeated 4 times. The group of negative controls (triplicate) contained additional water in place of the algae. An active control experiment (duplicate) was also performed to verify the activity of the inoculum, by using 1.1 g cellulose as the substrate instead of algae.

2.3. Sampling and sample analysis

The characteristics of the materials before and after stabilisation were analysed. During the stabilisation, a 1.5 ml liquid sample was taken from each reactor every one or two weeks. Total I, N, P and pH were measured in the liquid samples.

To monitor waste microalgae biomass degradation during aerobic stabilisation, the NaOH solutions in the exhaust line were analysed for CO_2 each time liquid was sampled from the reactors. After each sample, the exhaust gas traps were emptied and filled with fresh NaOH solution. For anaerobic stabilisation, a 6 ml gas sample from the headspace of each reactor was taken and measured while sampling the liquid.

Carbon and nitrogen content of the algae was analysed by a FLASH 2000 CHNS/O Analyser. Compositions of gas samples were analysed by a Shimadzu GC-2014. For analysing CO_2 produced by aerobic stabilisation, 1 ml NaOH solution from the exhaust gas traps was added to a sealed vacuum tube (12.5 ml capacity). Then each vacuum tube was filled with 4 ml of 4 M H_2SO_4 solution to ensure release of CO_2 under acidic conditions. The CO_2 content of the headspace of the vacuum tube was measured by GC.

In the aquatic environment, the species of elemental iodine vary as a function of pH and redox conditions (Gottardi, 2001). Also, soluble organic iodine compounds may be present. So to quantify the total iodine mobilised into the liquid phase, tetramethylammonium hydroxide (TMAH) was used to pre-treat the samples to ensure all the iodine species were converted to inorganic iodide (Fecher et al., 1998). 0.1 ml of filtered liquid sample was mixed with 0.1 ml 25% TMAH solution. The mixture was then heated in a water bath at 90 °C for three hours. After cooling, the liquid samples were then diluted by distilled water to 1 ml and then analysed by a Dionex ICS-3000 Ion Chromatography system with an AS-18 column. For the detection of iodine content in algae, wet biofilm samples were rinsed with distilled water and dried at 110 °C overnight. 0.2 g dry algal biofilm solid was digested following a procedure reported by Ródenas de la Rocha et al. (2009). After digestion, the iodine content in the liquid was measured by the ion chromatography method. Nitrogen and phosphorus content were monitored by Flow Injection Analysis (FIA).