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Cultivation of *Chlorella* on brewery wastewater and nano-particle biosynthesis by its biomass

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HIGHLIGHTS

- Nutrients in brewery wastewater were efficiently utilised by *Chlorella* sp. MM3.
- Substantial microalgal biomass obtained from brewery wastewater was used for iron nanoparticle synthesis.
- Algal biomolecules act as reducing and capping agents in iron nanoparticle synthesis.
- First report of nanoparticle synthesis using microalgae coupled with brewery wastewater remediation.

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ABSTRACT

This study investigated an integrated and sustainable approach for iron nanoparticles synthesis using *Chlorella* sp. MM3 biomass produced from the remediation of brewery wastewater. The algal growth characteristics, biomass production, nutrient removal, and nanoparticle synthesis including its characterisation were studied to prove the above approach. The growth curve of *Chlorella* depicted lag and exponential phase characteristics during the first 4 days in a brewery wastewater collected from a single batch of brewing process (single water sample) indicating the growth of algae in brewery wastewater. The pollutants such as total nitrogen, total phosphorus and total organic carbon in single water sample were completely utilised by *Chlorella* for its growth. The X-ray photoelectron spectroscopy spectra showed peaks at 706.56 eV, 727.02 eV, 289.84 eV and 535.73 eV which corresponded to the zero-valent iron, iron oxides, carbon and oxygen respectively, confirming the formation of iron nanoparticle capped with algal biomolecules. Scanning electron microscopy and particle size analysis confirmed the presence of spherical shaped iron nanoparticles of size ranging from 5 to 50 nm. To our knowledge, this is the first report on nanoparticle synthesis using the biomass generated from phycoremediation of brewery wastewater.

1. Introduction

Microalgae are biomolecules rich photosynthetic microorganisms, useful as an ecofriendly and economical biomass source in various fields including nanotechnology. Microalgal biomass contain varied proportions of protein (6–52%), carbohydrate (5–23%) and lipid (7–23%) (Brown et al., 1997), which involve in wide range of metal nanoparticle synthesis together with iron. This has been the case for the past few decades. Phyco-synthesis of iron nanoparticles is increasing in importance because they overcome the pitfalls of chemically synthesised nanoparticles such as instability, high cost involvement, and production of hazardous chemicals and solvents during synthesis. The polysaccharides and glycoproteins present in cell walls of microalgae reduce the bulk iron into nano-iron followed by capping, which results in highly stable and reactive nanoparticles. This is a most expected characteristic of the nanoparticles for their application in the field of contaminant remediation (Subramaniyam et al., 2015). However, considerable costs are involved in the algal mass production, because of the use of chemical fertilizers, energy and additional fresh water, which have restricted its application in practice. Nonetheless, it is essential to identify an alternative source for biomass production that is economically viable and eco-friendly.

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Recently, Renuka et al. (2015) reported that the biomass obtained as a result of phycoremediation of wastewaters can be put to use in cosmetics, pharmaceuticals, bioenergy production, animal and human feed production and bio-refinery. Wastewater effluents from industries contain nitrogen, phosphorous and heavy metals, which make water unsuitable for any beneficial uses, which is a major environmental concern. These wastewaters fulfil the growth requirements of algae resulting in the generation of biomass in an economical way, thus aiding in the process of phycoremediation of wastewater (Renuka et al., 2015). Successful application of microalgae grown in wastewaters has been employed for bioenergy production (Woertz et al., 2009; Lu et al., 2015; Kothari et al., 2012; Zhu et al., 2013). However, most of the wastewaters studied required some additional steps involving dilution of wastewater (Wang et al., 2015; Kothari et al., 2012; Zhu et al., 2013), adjusting pH (Lu et al., 2015; Wang et al., 2015), mixing of wastewaters (Lu et al., 2015) and providing additional sources including CO_2 (Woertz et al., 2009) to make it suitable for algal growth. This may be due to either very high nutrient concentration or unfavourable pH condition (acidic or alkaline). Moreover, the presence of heavy metals at higher concentrations may affect the algal growth and result in the deposition of these metals in the biomass produced, thus making it unsuitable for application.

In this regard, wastewater obtained from brewery industries will provide optimal conditions for algal growth with neutral pH and without any additional steps as it contains the minimum growth nutrients. The brewing industry consumes large amounts of water during the production process and 70% of it is released as wastewater (Valta et al., 2015). These wastewaters contain biodegradable and organic nutrients such as sugars, soluble starch, ethanol, volatile fatty acids, and very low concentrations of heavy metals (Driessen and Vereijken, 2003). Brewery wastewater as growth medium for algae has received less attention from researchers compared to other wastewaters such as piggeries, and the reason for this is the lower nutrient content of brewery wastewater. However, Raposo et al. (2010) reported a significant increase in ramified fatty acids and amino acid content (total and essential) in the biomass produced using brewery wastewater compared to biomass grown in normal nutrient medium. Furthermore, many studies have documented the inherent capacity of microalgae to grow under extreme conditions and especially concerning wastewaters (Woertz et al., 2009; Lu et al., 2015; Kothari et al., 2012; Zhu et al., 2013). Besides, the absence of significant amounts of heavy metals in the biomass produced using brewery wastewater is one of the most notable advantages for its downstream applications (Raposo et al., 2010).

It is evident from previous studies that among microalgae, *Chlorella* sp. has the inherent ability to adopt to most wastewaters as their growth medium (Woertz et al., 2009; Lu et al., 2015; Kothari et al., 2012; Zhu et al., 2013). Against this background, this study aims to unravel the potential of *Chlorella* sp. MM3 grown using brewery wastewater as the nutrient medium in iron nanoparticle synthesis. To our knowledge, this is the first report where the biomass obtained using wastewater is applied for nanoparticle synthesis. The objectives of the study are to: (i) test the growth of *Chlorella* sp. MM3 in brewery wastewater, (ii) remove nutrients from brewery wastewater by *Chlorella* sp. MM3, and (iii) synthesise and characterise iron nanoparticles using the above biomass.

2. Methods

2.1. Algal culture

Chlorella sp. MM3 (Accession No. JX126811) maintained axenically in the phycology laboratory of the Global Centre for Environmental Remediation (GCER), The University of Newcastle was used for this particular study. Stock cultures were maintained in Bold's basal medium and microscopically examined periodically for any signs of contamination (Megharaj et al., 1986).

2.2. Brewery wastewater

Two types of brewery wastewaters, namely single water sample and combined water sample present in Coopers Brewery, South Australia, were collected and employed for this study. Single water sample refers to fresh wastewater samples discharged from only a single batch of brewing process, while combined water sample is the collection of combined wastewater collected from several batches of brewery wastewater and stored for 24 h. These samples underwent the process of sedimentation and filtration (Whatman No. 45 filter paper) to remove large solid particles and stored at 4 °C and then used as and when required.

2.3. Screening of brewery wastewater

The two wastewater samples were screened for their characteristics and ability to serve as best growth medium for algal biomass production.

2.3.1. Characteristics of brewery wastewater

The characteristics including pH, total nitrogen, total phosphorous and total metals of these wastewaters were analysed. The samples were filtered, diluted and analysed in TOC–TN integrated analyzer equipment (TOC–VCSN–Total organic carbon analyzer, Shimadzu) for total nitrogen and total organic carbon (Mata et al., 2012). Similarly, total metals and total phosphorous were determined using Shimadzu inductively coupled plasma mass spectrometry (ICPMS).

2.3.1.1. Growth of Chlorella sp. MM3 in brewery wastewater. Initially, *Chlorella* sp. MM3 was found to be difficult to grow in both brewery wastewaters. Hence, the culture was allowed to acclimatise to both brewery wastewaters by exposing it to a mixture containing equal amounts of Bold's basal medium and brewery wastewater and the Bold's basal medium concentration was reduced. In the meantime, the brewery wastewater concentration was increased during every subculturing after a week of incubation. It was found that *Chlorella* sp. MM3 grew well in single water sample compared to combined water sample following three times subculturing. Once *Chlorella* sp. MM3 became acclimatised to both brewery wastewaters, 10 mL of well-grown algal culture was inoculated into 250 mL single and combined brewery wastewater contained in a 500 mL Erlenmeyer flask. The cultures were grown under continuous light of 200 μmol photons m^{-2} s^{-1} PPED at 25 ± 2 °C placed on an orbital shaker throughout the studies.

2.3.1.2. Algal growth determination. The growth of the acclimatised algae in both brewery wastewaters was checked periodically by measuring the optical density at 540 nm using a spectrophotometer (Genesys 5, Spectronic Instruments, UK). In addition, the optical density of algae grown in Bold's basal medium was also measured so that the growth of algae in brewery wastewater could be compared with Bold's basal medium. The brewery wastewater found to be more suitable for algal growth was considered for further studies.

2.4. Biomass production using phycoremediation

Based on brewery wastewater characteristics and algal growth, single water sample was taken for biomass production. As mentioned in Section 2.3.2.1, 10 mL of well-grown algal culture was inoculated into 250 mL single water sample and incubated under