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Review

Microalgal biomass for bioethanol fermentation: Implications for hypersaline systems with an industrial focus

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ABSTRACT

The potential of microalgae biomass as a feedstock for bioethanol fermentation has been widely considered in recent years. Yet, to date, only a modest level of research has been reported in this area. In all likelihood, the generation of a sustainable, sufficient level of biomass for biofuel production will need to be undertaken in saline water, and potentially under hypersaline conditions, to circumvent reliance on fresh water. However, the processing challenges associated with the fermentation of hypersaline biomass have yet to be adequately addressed. This review examines developments thus far for producing bioethanol from microalgae, indicating alternative means by which hypersaline microalgal biomass may be utilised, and provides a framework in which the industrial potential for sourcing such biomass should be considered.

1. Introduction

Over the last decade, the value of developing alternative sources for renewable liquid transportation fuels has increasingly been recognised. Key drivers for renewable energy have been the concern over global climate change associated with green house gas (GHG) emissions [1], as well as the speculation regarding energy security based on assessments that the world has already reached peak oil production [2]. To address the effective future mitigation of GHG emissions, the International Energy Agency (IEA) forecasted a 450 Scenario in their 2009 World Energy Outlook (WEO). This forecast, in which atmospheric GHG would be stabilised at a volume fraction of 450 μmol mol^{-1} carbon dioxide equivalent ($CO_2\cdot$eq), estimated that by 2030 the world demand for transport biofuels will be 11.64 EJ and supply 9.2% of total global transport fuels. This is equivalent to an annual production growth of 9.6% from a 1.43 EJ global biofuel output in 2007 [3]. Two important transport

Abbreviations: afdw, ash free dry weight; dw, dry weight; GHG, green house gas; PBR, photobioreactor; $CO_2\cdot$eq, carbon dioxide equivalent; w_B, mass fraction of B (mass of substance B divided by the mass of the mixture): $w_B = m_B/m$.

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biofuels are bioethanol and biodiesel. Of these, bioethanol is currently produced in the largest volumes; for example, the bioethanol component of worldwide biofuel production in 2007 was 1.06 EJ, equivalent to 5.0×10^{10} dm^3 [4]. In the automotive industry, bioethanol has emerged as the primary alternative renewable transport fuel to supplement and potentially replace gasoline [5], and is, additionally, under consideration as a low-blend additive to diesel [6,7].

Bioethanol derived from photosynthetic organisms can mitigate the impact of GHG emissions through photoautotrophic conversion of atmospheric carbon dioxide to useful biomass. However, first generation bioethanol made from starch-rich agricultural produce have received widespread criticism for being unsustainable and socially irresponsible [8]. These concerns have led to development of second generation bioethanol from more sustainable feedstocks, such as cellulosic biomass [9]. Even so, proponents for the impact of global land use change (LUC) continue to question the overall benefit that biofuels, such as current bioethanol and biodiesel, derived from land vegetation will have on net global GHG emissions [10]. Furthermore, there remains uncertainty as to how future governmental policies on biofuels might be impacted with regard to the consequences of indirect land use change (iLUC) and its associated costs [11].

The potential for producing third generation bioethanol from alternative biomass such as microalgae and macroalgae has generated significant interest. A comprehensive recent review of this topic by John *et al*. (2011) [12] acknowledged a need for high salt tolerant microalgal species to improve utilisation of seawater. Marine or hypersaline algal species are desirable as sustainable sources of biomass; however, the fundamental aspect of hypersalinity inherent with such biomass is under-emphasised and has not been addressed in the majority of research reported in this area. Microalgae and macroalgae are often considered together when compared to terrestrial sources of biomass, yet they each present very different processing challenges from farming, production and conversion. Marine microalgae targeted for biofuels could be harvested from open or closed mass culturing systems, and most likely operated under hypersaline conditions to conserve water. Additionally, for both practical and economical reasons, marine microalgae as viable biofuel feedstocks cannot be completely dewatered and washed; hence concentrates of harvested marine microalgal biomass would carry significant quantities of saline or hypersaline water. In contrast, marine macroalgae would commonly be harvested from their natural coastal environment, whether wild or farmed, and can be more easily dewatered and washed.

There has been significant recent progress in the fermentation of marine macroalgal biomass for biofuel production. For instance, an important breakthrough microbial platform has been engineered in *Escherichia coli* for simultaneous saccharification and fermentation (SSF) of alginate, and enhanced assimilation of mannitol and glucose to bioethanol from the brown macroalga *Saccharina japonica* [13]. Additionally, there is now considerable research and commercial interest in developing marine macroalgae as a feedstock for biobutanol production through acetone butanol ethanol (ABE) fermentation with *Clostridia* spp. [14,15]. The conversion of marine macroalgal biomass to bioethanol and biobutanol warrants review to assess the implications of recent developments; particularly for macroalgal biomass conversion to biobutanol which has a higher energy density and better gasoline engine compatibility than bioethanol [16]. However, the review reported here will focus primarily on marine microalgae as a feedstock for bioethanol in order to highlight the importance of hypersaline systems for consideration.

While the attention of many research groups has focused towards utilising marine microalgal biomass for sustainable energy supplies, there has been minimal research and development on biofuel production under hypersaline conditions. In many parts of the world where arable land and fresh water resources are limited due to marginal climate conditions or demands associated with population growth, hypersaline processes will likely be required for sustainable commercial biofuel production from microalgae, particularly at the scale needed to supplement or supply the world's insatiable appetite for liquid transportation fuels [17]. The concerns around sustainable use of land and fresh water resources can be obviated through the development of marine based systems for producing biofuels. Photosynthetic marine microalgal production systems are well suited to this purpose as the microalgae can be cultured using seawater in arid locations that have no agricultural value. Microalgae are resilient organisms and include many culturable species rich in lipids and carbohydrates. They grow rapidly and are able to produce a daily average 20 g m^{-2} ash free dry weight (afdw) biomass in open raceway pond systems, making them ideal biomass feedstock for producing biofuels [17]. This average microalgal biomass productivity is equivalent to an annual yield of 7.3 Mg km^{-2} and is comparable to that of sugarcane crops, which have been reported at between 7.0 Mg km^{-2} to 7.7 Mg km^{-2}; the largest energy crop harvested for bioethanol [5,18]. Indeed, it is anticipated that microalgae will be an important biomass for third generation biofuels. Although almost all of the reported research on microalgal liquid biofuels is focused on the production of lipid for biodiesel [19], the economic feasibility of generating biodiesel from a microalgal biomass feedstock may depend on the development of energy co-products such as methane, hydrogen, ethanol, butanol and aviation fuel [20].

In the early 1980s, it was recognised that microalgae such as the genus *Dunaliella* had potential as a rich, renewable biomass for use in the fermentative production of butanol and ethanol [21]. Furthermore, the increased global pressure on the resource sector to produce fuel from renewable and environmentally sustainable sources has led to ongoing research in methods to produce ethanol from microalgae since the late 1990s [12,22–27]. As a result, a modest body of knowledge now exists that demonstrates the feasibility of two main approaches for producing ethanol from microalgae. One area gaining significant interest is the anaerobic fermentation of the microalgal biomass by solventogenic microorganisms, which can utilise and convert the microalgal carbohydrates to ethanol. Another field of research has demonstrated the direct synthesis of ethanol as a metabolite in strains of both naturally occurring and engineered microalgae. Some such processes are currently under commercial development [28,29], although bioethanol made from microalgae is not yet available on the market.