ARTICLE IN PRESS

ALGAL-00747; No of Pages 21

Algal Research xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Review of the cultivation program within the National Alliance for Advanced Biofuels and Bioproducts

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ARTICLE INFO

Article history:
Received 18 March 2015
Received in revised form 21 October 2016
Accepted 28 November 2016
Available online xxxx

ABSTRACT

The cultivation efforts within the National Alliance for Advanced Biofuels and Bioproducts (NAABB) were developed to provide four major goals for the consortium, which included biomass production for downstream experimentation, development of new assessment tools for cultivation, development of new cultivation reactor technologies, and development of methods for robust cultivation. The NAABB consortium testbeds produced over 1500 kg of biomass for downstream processing. The biomass production included a number of model production strains, but also took into production some of the more promising strains found through the prospecting efforts of the consortium. Cultivation efforts at large scale are intensive and costly, therefore the consortium developed tools and models to assess the productivity of strains under various environmental conditions, at lab scale, and validated these against scaled outdoor production systems. Two new pond-based bioreactor designs were tested for their ability to minimize energy consumption while maintaining, and even exceeding, the productivity of algae cultivation compared to traditional systems. Also, molecular markers were developed for quality control and to facilitate detection of bacterial communities associated with cultivated algal species, including the *Chlorella* spp. pathogen, *Vampirovibrio chlorellavorus*, which was identified in at least two test site locations in Arizona and New Mexico. Finally, the consortium worked on understanding methods to utilize compromised municipal wastewater streams for cultivation. This review provides an overview of the cultivation methods and tools developed by the NAABB consortium to produce algae biomass, in robust low energy systems, for biofuel production.

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http://dx.doi.org/10.1016/j.algal.2016.11.021

1. Introduction

1.1. Preface

Humans have marveled at the complexity and multiplicity of aquatic microorganism since drops of pond water were first examined under a microscope. Photosynthetic microorganisms were necessarily classified based on morphological differences in the descriptive era of biological science. Over time, chemical and biochemical characteristics began to take on more taxonomic significance as expounded by R.Y. Stanier [1] and more recently by others [2–3]. Even the notion of "species" as applied to microalgae has been reexamined recently with specific reference to the diatoms. The result was a more holistic approach to taxonomy, integrating biochemical, molecular and ecological characteristics that suggests the species concept has been applied far too broadly within the microalgae [4]. In sum, these studies provide a critically important backdrop to the current era. We know that primary production in aquatic systems is highly competitive and unstable in a biological sense [5]. Constant changes in nutrient levels, light intensity and temperature will trigger winners and losers in the competition for available light, inorganic carbon, nitrogen and phosphorus. Morphologically similar species with very different metabolisms will wax and wane with seasons and on more rapid time scales dictated with rainfall, wind, dust and diurnal temperature ranges. Large-scale microalgal cultivation systems will need to be designed to mitigate all of these ecological eventualities. Crop protection strategies against grazers [6], pathogens [7,8] and competitors [9,10] will be equally important.

Conceptually, the National Alliance for Advanced Biofuels and Bioproducts (NAABB) team was strongly influenced by the opportunities made available by the radical successes of reductionist biology: complete genome sequences, multiple "omic" analytical tools and detailed structure/function studies that provide both methods and metabolic insights needed for genetic manipulation of model microalgae. These tools provide tantalizing approaches to rapidly domesticate and improve wild microalgal species for renewable fuel production centered on neutral lipid synthesis [11] and secretion of pure hydrocarbons [12,13]. Well-funded start-up enterprises both within and outside the NAABB consortium set out to harvest this bounty of opportunity. Tightly coupled with this approach is the assumption that monocultures of elite algal strains can be effectively maintained in appropriately engineered cultivation systems. Modern agriculture provides a compelling model based on monocultures afforded by powerful methods of crop protection.

It is important to note that this approach has been questioned by two key studies: the final report of the DOE Aquatic Species Program (ASP) [14] and more recently in the report on Algal Biofuels from the National Research Council [15]. The ASP report cited repeated difficulties with maintaining desired strains in open raceway cultivation systems and went so far as to suggest better results might be obtained by cultivation of highly competitive local species with desired phenotypes over the use of elite strains of microalgae isolated elsewhere. The NRC study cites broader concerns regarding unsustainable requirements for energy, water and nutrients for elite genetically modified algae at scales required for production of 5% of the nations liquid fuel requirements. The NRC report provides important high-level guidance for future studies by identifying the significant barriers to sustainable algal biofuel production.

1.2. Approach

The NAABB cultivation studies outlined here reflect key design criteria with respect to large-scale cultivation. It was important to identify and select geographic locations that have high annual solar insolation and climatic conditions that can maintain pond water temperatures at elevated levels for most of the year. The NAABB cultivation teams attempted to identify those microalgae strains that exhibited high growth rates within the annual temperature range and water chemistries from the production sites. Also, there was a need to design relatively simple, cost-effective, and energy-efficient large-scale culture systems that could support high productivity, culture stability and help maintain elevated water temperatures during the cold season to sustain reasonable biomass productivities year round. Specific goals included the following:

- identify robust production strains that will perform reliably in specific geographic locations and seasons;
- develop methods and best practices for preventing large-scale culture crashes due to predators and competitors;
- develop methods for cultivation in low-cost media using agricultural grade nutrients, wastewater sources, and media recycling; and
- develop and demonstrate enhanced designs and operational methods that improve productivity of large-scale cultivation systems.

In response, the NAABB cultivation team executed the projects reviewed here. Highlights include publications that: i) demonstrated an effective raceway design (ARID) for temperature management in modified raceway systems [16–18]; ii) studied the energy efficiency of different cultivation systems [19–28]; iii) developed sensitive methods for detection of both closely and distantly related algal competitor strains and used these to monitor long-term cultivation [29,30]; iv) created detailed algal growth models for a *Scenedesmus* strain [31] as well as the most productive (*Chlorella sorokiniana* DOE1412) and stable (*Nannochloropsis salina*) organisms used by the NAABB consortium [32]; v) evaluated polyculture approaches to increasing pond productivity, stability and resilience [33]; and vi) identified a new approach to cultivation using extreme conditions of low pH and high temperature appropriate for evaporation control in photobioreactors [34,35].

The NAABB Consortium developed an R&D framework to begin addressing some of these major challenges and needs. The NAABB Cultivation team employed a variety of capabilities to execute the R&D framework. These include research tools (photobioreactors and specialized laboratory growth systems), small-pond/raceway testbeds and two large-pond testbeds for large-scale cultivation experiments and the production of algal biomass to support downstream processing R&D across NAABB [36].

As shown in Fig. 1, NAABB cultivation research was conducted across four major thrust areas:

- Cultivation Tools and Methods: Focused on developing new strain screening tools, systems, and models; sensors for cultivation; molecular diagnostics tools; and methods to control predators with environmental controls.
- Nutrient/Water Recycle/Wastewater Cultivation: Focused on nutrient studies, use of wastewater sources, and media recycle.
- Cultivation System Innovations: Focused on new raceway design to extend operation in cold season climates, airlift mixing systems, and computational fluid dynamic (CFD) models to improve raceway performance.
- Large-pond Cultivation/Biomass Production: Focused on scale-up of new strains, development of low-cost media, and production of algal biomass.
- Measured the productivity of different strains at testbeds using open raceway and photobioreactor systems

2. Technical accomplishments

2.1. Cultivation tools and methods

NAABB focused several efforts on developing new tools and methods for optimizing algal cultivation. These include new models and processes to select strains and cultivation conditions for maximum