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A process economic assessment of hydrocarbon biofuels production using chemoautotrophic organisms

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HIGHLIGHTS

- Process model presented for microbial hydrocarbon production from H_2, O_2 and CO_2.
- Economic analysis is used to obtain capital, operating cost and fuel cost estimate.
- Electricity cost is found to be >90% of fuel cost.
- Specific fuel productivity target $\geqslant$0.3 g-fuel/gDW-h is needed for feasibility.
- Economic feasibility requires LCOE <2¢/kWh LCOE for target specific productivity.

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ABSTRACT

Economic analysis of an ARPA-e Electrofuels (http://arpa-e.energy.gov/?q=arpa-e-programs/electrofuels) process is presented, utilizing metabolically engineered *Rhodobacter capsulatus* or *Ralstonia eutropha* to produce the C_{30+} hydrocarbon fuel, botryococcene, from hydrogen, carbon dioxide, and oxygen. The analysis is based on an Aspen plus® bioreactor model taking into account experimentally determined *Rba. capsulatus* and *Rls. eutropha* growth and maintenance requirements, reactor residence time, correlations for gas–liquid mass-transfer coefficient, gas composition, and specific cellular fuel productivity. Based on reactor simulation results encompassing technically relevant parameter ranges, the capital and operating costs of the process were estimated for 5000 bbl-fuel/day plant and used to predict fuel cost. Under the assumptions used in this analysis and crude oil prices, the Levelized Cost of Electricity (LCOE) required for economic feasibility must be less than 2¢/kWh. While not feasible under current market prices and costs, this work identifies key variables impacting process cost and discusses potential alternative paths toward economic feasibility.

1. Introduction

Fossil fuels have provided a readily accessible and energy-dense fuel source to pave the way for science and technology advancements. However, fossil fuel reservoirs, which accumulated over millions of years, are a finite resource that must be replaced with more sustainable alternative liquid fuels. Corn ethanol, the most widely produced biofuel in the United States, made up less than 5% of transportation fuels in 2011 (www.eia.gov). Furthermore, there is ongoing controversy related to corn-ethanol contributing to higher food prices. Additional constraints on alternative fuel technologies include valuation of carbon credits and assurance of economic and political energy security. These competing issues demand the development of transformative technologies for producing renewable liquid fuels able to meet our society's growing energy needs.

The Electrofuels initiative, administered by the United States Department of Energy's Advanced Research Projects Agency – Energy (ARPA-E), aimed to develop liquid biofuels that avoid the issues encountered in the current generation of biofuels: (1) the reliance of biomass-derived technologies on the inefficient process of photosynthesis, (2) the relatively energy- and resource-intensive nature of agronomic processes, and (3) the occupation of large areas of arable land for feedstock production (http://arpa-e.energy.gov/?q=arpa-e-programs/electrofuels). To address these issues, the

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List of symbols and abbreviations

a, b, c, d	stoichiometric coefficients in cell growth equation
bbl	US fluid barrel
C_{fuel}	concentration of fuel in the bioreactor, mol L^{-1}
C_i	liquid phase concentration of i-th gas component, mol L^{-1}
D_i	diffusion coefficient of i-th gas component, m^2 s^{-1}
$\frac{dc_i}{dt}$	rate of consumption of i-th gas component, mol L^{-1} h^{-1}
$\left[\frac{dc_i}{dt}\right]_{growth}$, $\left[\frac{dc_i}{dt}\right]_{fuel}$, $\left[\frac{dc_i}{dt}\right]_{maint}$, $\left[\frac{dc_i}{dt}\right]_{total}$	rate of substrate utilization for growth, fuel synthesis, maintenance requirements and total respectively, mol L^{-1} h^{-1}
EROI	Energy Return on Investment
F	rate of liquid feed into reactor, L h^{-1}
gDW	grams dry weight
GTR_i	gas transfer rate of i-th gas component, mol L^{-1} h^{-1}
H_i	Henry's law coefficient of i-th gas component, atm L mol^{-1}
IGCC	integrated gasification combined cycle
K_La_i	gas–liquid mass transfer coefficient of the i-th component, h^{-1}
LCOE	Levelized Cost of Electricity
m_{H_2}, m_{O_2}	maintenance coefficient of cells on H_2 and O_2 respectively mol gDW^{-1} h^{-1}
NGCC	natural gas combined cycle
PC	pulverized coal
$\bar{P}_f$	volumetric fuel productivity, g-fuel L^{-1} h^{-1}
P_{tot}	total pressure, atm
R_{fuel}	specific fuel productivity, g-fuel·gDW^{-1} h^{-1}
r_{growth}	rate of growth of cells, gDW L^{-1} h^{-1}
V	reactor volume, L
X	cell density, gDW L^{-1}
Y_{f/H_2}	yield of fuel on H_2, g-fuel·$(mol\text{-}H_2)^{-1}$
y_i	gas phase mole fraction of i-th component
$Y^T_{H_2}$	true growth yield of cells on H_2, gDW mol^{-1}
ε	cell recycle efficiency
μ, μ_{max}	specific growth rate and maximum specific growth rate of cells, h^{-1}
τ	residence time through reactor, V/F, h^{-1}

Electrofuels initiative funded research efforts that sought to develop biological processes that would convert distributed, off-grid, renewable electricity into alternative liquid fuels. The logic of this approach rests in its ability to provide a reliable energy source for the transportation sector by storing transiently-available electrical energy in a chemical bond (Fig. 1A). In addition, the Electrofuels approach is synergistic with advances in photovoltaic cells.

Under the Electrofuels initiative, a range of approaches to this challenge were funded (summary found in (Tuerk, 2011)). The concept for our approach is depicted schematically in Fig. 1A, and involves collecting and transporting electrons to a centralized bioreactor for biological capture of the reducing power in the chemical bonds of a hydrocarbon fuel. This proceeds by: (1) the capture of solar energy into electrical energy via photovoltaic cells (with demonstrated laboratory efficiencies upwards of 40%, a sevenfold improvement on photosynthesis), (2) the use of the generated electricity to split water into molecular hydrogen (H_2) and oxygen (O_2), and (3) feeding these gases, along with carbon dioxide (CO_2) emitted from point sources such as a biomass or coal-fired power plant, to a microbial bioprocessing platform. Our proposed microbial bioprocessing platform leverages a chemolithoautotrophic microorganism naturally able to utilize these gases as growth substrates, and genetically modified to produce a triterpene hydrocarbon fuel molecule native to the alga *Botryococcus braunii*. The details and rationale of these choices are discussed below.

This exercise of using process economic analyses to research priorities is an important aspect of the ARPA-E program. An initial analysis of the economic feasibility of the microbial process, based on microbial energetic theory, can be found in an extensive thesis (Tuerk, 2011). A preliminary process economic model can also be found in a co-author's honors thesis (Myers, 2013). In this work, we expand upon these previous analyses by constructing a more detailed bioreactor model in Aspen Plus® (described in Section 2.2). Furthermore, we examined specific scenarios based on reactor residence time (τ), specific fuel productivity (R_{fuel}) and gas–liquid mass transfer coefficient (k_La) to predict their effect on the overall process volumetric fuel productivity ($\bar{P}_f$) and fuel cost ($/bbl-fuel). The ultimate goal of this analysis was to identify limiting parameters for the process and ranges of these variables needed to achieve economic feasibility.

1.1. Rationale for selection of the microbial bioprocessing platform

Biological approaches for producing alternative fuels are benefitting from advances in molecular biology. These developments have increased the range of fuel molecule targets that can be synthesized by living organisms (Farmer and Liao, 2001; Kim et al., 2006), as well as expanded the selection of alternative hosts for production through the development of new genetic engineering tools (Steinbrenner and Sandmann, 2006). However, current works most frequently uses *Escherichia coli* or yeast as the microbial host with carbohydrate (i.e. fixed-carbon) based substrates. Our approach to the Electrofuels initiative leverages developments in alternative hosts capable of autotrophic growth on H_2 as well as developments in fuel targets.

1.1.1. Microbial host selection

For this economic analysis, we decided to include two different chemolithoautotrophs (microbes growing on H_2, O_2 and CO_2) based on specific differences in their physiology, metabolism and energetic yields. Initially motivated by the production of the industrially relevant biopolymer poly-hydroxy butyrate (PHB), the chemoautotrophic growth mode was developed as an alternative approach to bioprocessing using *Ralstonia eutropha* (Tanaka et al., 1995). High-density growth (40–90 gDW/L) of *Rls. eutropha* on gaseous substrates was achieved in high gas–liquid-mass-transfer bioreactors with controlled addition of inorganic nutrients, demonstrating the technical feasibility of such a process. We are also investigating the use of *Rhodobacter capsulatus*, a purple non-sulfur facultative phototroph, as a candidate because of its capability of diverse metabolic modes (including autotrophic), ability to utilize a range of growth substrates, and innate high level production of carotenoids (Hunter et al., 2009) indicating that pathways for isoprenoid biosynthesis are already present in this organism. We have recently achieved significant milestones, including production of >100 mg/L botryococcene, by genetically engineering *Rba. capsulatus* (Khan et al., 2013), validating its poten-

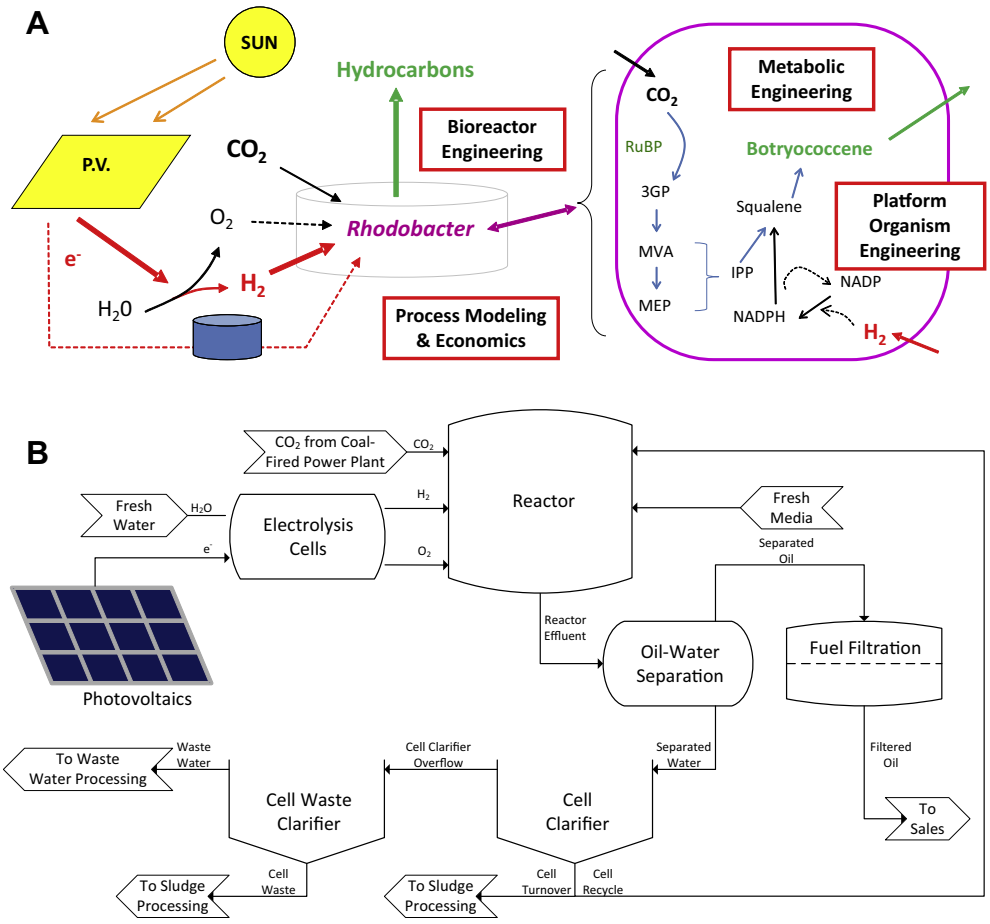

Fig. 1. (A) Conceptual depiction of an Electrofuels process. Energy from the sun is captured in the form of renewable electricity (depicted as solar photovoltaics, PV) and is used to split water. The resulting O_2 and H_2 is combined with CO_2 and fed into a bioreactor where *Rba. capsulatus* consumes these gases in stoichiometric requirement. *Rba. capsulatus* is genetically engineered to produce a C_{30+} triterpene hydrocarbon which can be recovered as an extracellular phase-separated oil. (B) Process flow diagram of the envisioned Electrofuels process.

tial as an alternative host for isoprene based fuels and molecules. As will be discussed later, *Rba. capsulatus* is less efficient in utilizing H_2 for growth, but has lower maintenance requirements than *Rls. eutropha*, differences which provide economic tradeoffs in an Electrofuels process. The range of yield and maintenance parameters between *Rba. capsulatus* and *Rls. eutropha* is likely representative of most chemoautotrophs and we were interested in quantifying how these contrasting energetic needs affect the overall economics of the process.

1.1.2. Hydrocarbon fuel molecule selection

One of the largest barriers faced by alternative energy technologies is poor economics relative to fossil fuels. Having been the most widely developed biofuel to date, ethanol has achieved the most success competing economically with gasoline. However, ethanol's relative competitiveness results in part from governmental subsidies and mandates that ensure lower cost of ethanol feedstocks and higher selling prices (Solomon et al., 2007). Estimates of the Energy Return on Investment (EROI) of corn-derived ethanol currently range from a mere 0.7 to 1.65, while those estimated for cellulosic ethanol range from 4.40 to 6.61 (de Castro et al., 2014; Hammerschlag, 2006).

We have chosen the hydrocarbon botryococcene as the product of the Electrofuels process as a means of addressing issue of energy-intensive industrial processing, which is a major drawback to ethanol production. In choosing this fuel molecule target, the project leveraged our experience in working with the alga *B. braunii* (Khatri et al., 2014) and the associated discovery of the enzymes responsible for the synthesis of C_{30} squalene and botryococcene (Okada et al., 2004). C_{30+} triterpenes include methylated botryococcenes and squalenes, and are produced by *B. braunii* race B, a colonial green algae that accumulates these hydrocarbon oils up to 30% of its dry weight composition (Metzger and Largeau, 2005). Ancestors of this alga have been implicated in producing up to 1.4% of the total hydrocarbon in maar-type oil shales based on the unique carbon footprint of C_{34} botryococcene oil (Derenne et al., 1997). They have properties similar to crude oil (Hillen et al., 1982), a higher energy density than ethanol (38.1 vs. 23.5 kJ/L) (Tuerk, 2011) and can be easily separated from an aqueous phase by decantation (Khatri et al., 2014). Given these advantages, it was anticipated that the production of botryococcenes could yield higher EROI than ethanol.

2. Methods: model development

2.1. Proposed electrofuels production process

The process flow diagram of the proposed Electrofuels production process is depicted in Fig. 1B. Production of the C_{30+} hydrocarbon fuel by the host organism occurs in the bioreactor, which is fed the gaseous substrates H_2, O_2 and CO_2. The relative amount of these gases fed is equal to the ratio that supports growth and fuel production by the bacteria. The determination of this stoichiometric ratio is discussed in further detail under the Section 2.2.3.

Other nutrients required for cell growth, such as salts and vitamins, are fed to the bioreactor via a liquid stream. The bioreactor is operated continuously and the bioreactor effluent contains biomass, botryococcene fuel product, and spent culture medium. An underlying assumption critical to this process configuration is that the botryococcene fuel is excreted and deposited as a hydrophobic layer on top of the culture, which can be separated by decantation (oil–water separator). The decanted hydrocarbon layer then flows from the oil–water separator to a fuel filtration system where the residual aqueous phase is removed, and the fuel molecules can then exit the process as the crude hydrocarbon product. We did not include subsequent processing, such as hydrocracking, within the scope of this economic analysis, since the point of comparison is crude oil, which would require similar downstream processing (Hillen et al., 1982).

The aqueous phase that exits the oil–water separator flows to a cell clarifier. This concentrates the biomass phase before returning a portion of the biomass to the bioreactor, and the remaining biomass to sludge processing to avoid accumulation of dead cells and cellular waste (perfusion mode operation). The spent media passes through a waste treatment process to remove cellular debris and waste material, and is recycled as fresh medium after supplementing with salts and other nutrients.

2.2. Bioreactor modeling

The biological processes taking place in the bioreactor (cell growth, maintenance and fuel molecule synthesis) determine the rate of substrate consumption (H_2, CO_2, O_2), and therefore the gas–liquid mass-transfer (k_La) requirements, which are the most critical components of the bioreactor operating cost. The bioreactor volumetric fuel productivity (dependent on the specific fuel productivity, residence time and k_La) and the scale of the process (bbl-fuel/day) determine the required size of all associated process equipment, and thus the capital and fixed costs. The capital investment at that plant size can then be amortized at a nominal internal rate of return and combined with the operating costs to arrive at an estimated cost for crude fuel production ($/bbl-fuel). A detailed model of the bioreactor processes associated with hydrocarbon fuel production, which includes cell growth and maintenance, fuel synthesis, and gas transport, is therefore a critical prerequisite to accurately estimating the capital and operating costs.

In aerobic chemolithoautotrophic metabolism (referred to as 'autotrophic' metabolism from here on), electrons are transferred from H_2 to O_2 through a series of steps which generate the energy needed for various cellular processes, including CO_2 fixation for growth and fuel synthesis. Thermodynamic models (McCarty, 2007; McCarty, 1971; Tuerk, 2011) can be used to calculate these requirements. The microbial substrate requirements ultimately reduce to stoichiometric equations that can be incorporated in a bioreactor model. To integrate the bioreactor model with the rest of the process, we used Aspen plus® software. We selected Aspen plus® as it can solve complicated material and energy balance equations with rigorous physical property determinations, and we selected the RCSTR reactor model to incorporate the microbial energetic equations described below in Sections 2.2.1 through 2.2.4.

2.2.1. Substrate requirement for cell growth

Fig. 2A depicts the conceptual framework for modeling cell growth and maintenance processes. In this context, growth processes refer to the use of energy and carbon substrates for the production of new cellular biomass, whereas cellular maintenance is the use of energy substrates for non-growth processes such as motility, ion pumping, futile cycling etc. (Pirt, 1965; van Bodegom, 2007). For autotrophic metabolism, the process of cell

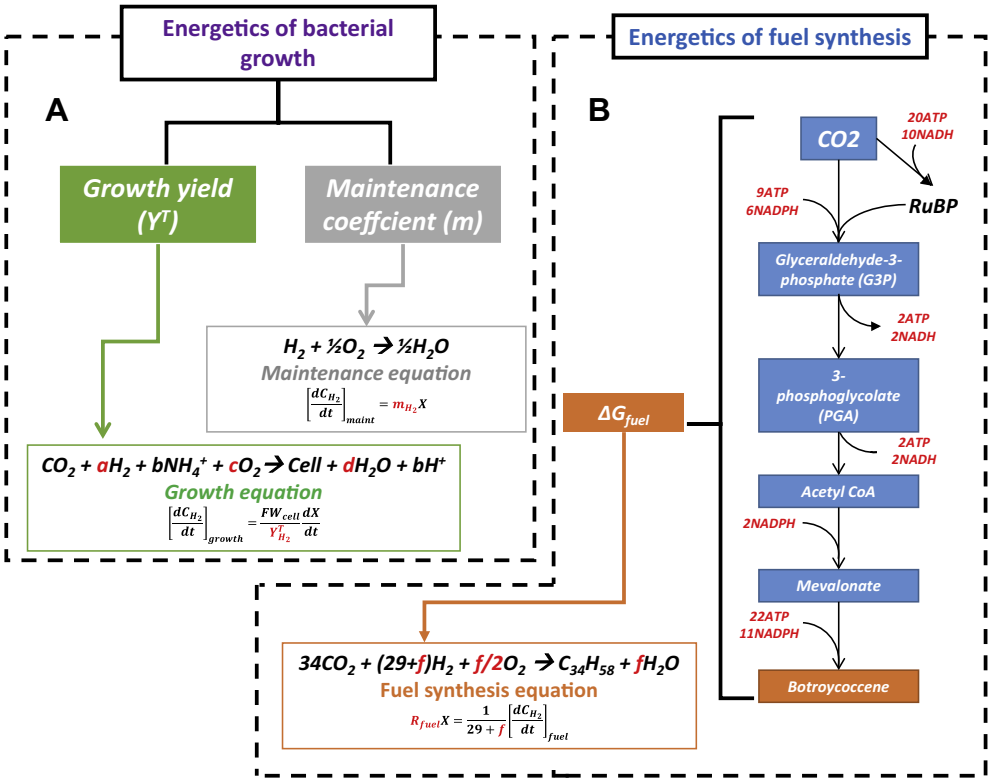

Fig. 2. Framework for capturing microbial growth energetics, cellular maintenance and fuel production. (A) Incorporating the experimentally measured biological yield and maintenance coefficients into model reaction/stoichiometry equations suitable for reactor design. (B) Concise version of metabolic pathway for calculating the minimum required Gibbs' free energy for fuel synthesis in a bacteria.

growth (excluding energy demands for maintenance) can be represented by a stoichiometric relationship, the 'growth equation' as shown below in Eq. (1):

$$CO_2 + aH_2 + bNH_4^+ + cO_2 \rightarrow \text{Cell}\left[CN_bH_{(2a+3b-2d)}O_{(2+2c-d)}\right] + dH_2O + bH^+ \quad (1)$$

In this relationship, the inorganic carbon source (CO_2), electron donor (H_2), electron acceptor (O_2), and nitrogen source (ammonium, NH_4^+) are consumed, resulting in the formation of cell biomass, water (H_2O), and hydrogen ions (H^+). Consequently, the rate of consumption of each of the gases due to growth processes $\left(\left[\frac{dC_i}{dt}\right]_{\text{growth}}, \text{ where } i \text{ represents } H_2,\ CO_2,\ \text{or } O_2\right)$ is related to the rate of cell growth $\left(\frac{d[X]}{dt}\right)$ by Eq. (2),

$$r_{\text{growth}} = \frac{d[X]}{dt} = \frac{1}{a}\left[\frac{dC_{H_2}}{dt}\right]_{\text{growth}} = \frac{1}{c}\left[\frac{dC_{O_2}}{dt}\right]_{\text{growth}} = \left[\frac{dC_{CO_2}}{dt}\right]_{\text{growth}} \quad (2)$$

The indices for the elements N, H and O in the cell biomass term of Eq. (1) [b, $2a+3b-2d$ and $2+2c-d$, respectively] were obtained from literature reports of the empirical elemental formula for cell biomass for each *Rba. capsulatus* and *Rls. eutropha* (Hoekema et al., 2006; Ishizaki and Tanaka, 1990), resulting in three of the four equations needed to calculate the four coefficients a, b, c and d. The fourth equation needed to complete the set is from the true growth yield (substrate requirement for growth that could occur in the absence of cellular maintenance requirements) of the bacteria on the energy substrate H_2 ($Y^T_{H_2}$) and is related to the coefficient a by Eq. (3),

$$Y^T_{H_2} = \frac{FW_{\text{cell}}}{a} \quad (3)$$

where FW_{cell} is the formula weight of one 'mole' of cell according to the empirical formula for cell biomass. Because $Y^T_{H_2}$ is the quantity of H_2 needed for growth-only processes (and does not include energy requirements for maintenance), this parameter can be obtained through the use of microbial energetic models (Tuerk, 2011) or experimental measurements (Pirt, 1965).

2.2.2. Substrate requirement for cellular maintenance

Additional consumption of H_2 and O_2 occurs in order to generate energy for cellular maintenance. Because cellular maintenance requirements are defined as independent of energy requirements for growth, the simplest way to conceive of these requirements is to consider them on a per-time, per-mass of cell basis, and thus the maintenance coefficients (m_{H_2} and m_{O_2}) are a measure of the rate of substrate required to satisfy the maintenance needs of one unit mass of cells. The rate of consumption of H_2 and O_2 due to cellular maintenance is therefore proportional to the total biomass concentration in the reactor, X, as shown in Eq. (4):

$$m_{H_2}X = \left[\frac{dC_{H_2}}{dt}\right]_{\text{maint}} = \frac{1}{2}m_{O_2}X \quad (4)$$

m_{O_2} is related to m_{H_2} by a factor of two because of the stoichiometry of water formation, which is the net reaction for energy generation in autotrophic metabolism since electrons from H_2 are transferred to O_2. We initially presented values of $Y^T_{H_2}$ and m_{H_2} based on literature reports and used them in conjunction with microbial energetic theory to predict net growth and fuel yields under variety of conditions (Tuerk, 2011). To confirm these estimates towards improving the accuracy of this economic analysis, we have recently measured the values of $Y^T_{H_2}$ and m_{H_2} under identical conditions for both *Rba. capsulatus* and *Rls. eutropha* using an adaptation of the traditional methods using continuous chemostat cultures under autotrophic conditions (Pirt, 1965).

2.2.3. Substrate requirement for fuel synthesis

To incorporate botryococcene fuel synthesis into the bioreactor model, we first defined a parameter, R_{fuel}, the specific fuel productivity. The rate of fuel synthesis is therefore given by Eq. (5):

$$\left[\frac{dC_{\text{fuel}}}{dt}\right]_{\text{fuel}} = R_{\text{fuel}}X \quad (5)$$

The botryococcene fuel molecule, $C_{34}H_{58}$, is energetically expensive to synthesize. Therefore, additional H_2 and O_2 must be consumed to provide energy for fuel synthesis, beyond the requirements for cell growth. We designate the parameter f to account for the additional H_2 which must be oxidized in order to provide this energy; this is above and beyond the hydrogen incorporated into the botryococcene molecules themselves. The overall fuel synthesis reaction is summarized in Eq. (6). This treatment is analogous to cell growth and maintenance described in Sections 2.2.1 and 2.2.2:

$$34CO_2 + (29+f)H_2 + \frac{f}{2}O_2 \rightarrow C_{34}H_{58} + fH_2O \quad (6)$$

The minimum energetic requirement for botryococcene synthesis from CO_2 via the Calvin–Benson–Bassham (CBB) and mevalonic acid (MVA) pathways can be calculated by summing the total Gibbs energy required for generating the cofactors (mainly ATP, NAD(P)H and NADH) in the metabolic steps. A simplified version of the relevant metabolic pathways is shown in Fig. 2B. The cofactor requirement for going from mevalonate to botryococcene has been previously elucidated by (Okada et al., 2004). The total Gibbs energy required for synthesizing one mol of botryococcene from CO_2 was converted to the amount of H_2 (f in Eq. (6)) that must be oxidized to provide this energy. The rate of consumption of H_2, O_2 and CO_2 for fuel synthesis can then be calculated through Eq. (7),

$$R_{\text{fuel}}X = \frac{1}{29+f}\left[\frac{dC_{H_2}}{dt}\right]_{\text{fuel}} = \frac{2}{f}\left[\frac{dC_{O_2}}{dt}\right]_{\text{fuel}} = \frac{1}{34}\left[\frac{dC_{CO_2}}{dt}\right]_{\text{fuel}} \quad (7)$$

The fuel mass yield on H_2 (Y_{f/H_2}) is defined as the quantity $\frac{FW_{\text{fuel}}}{29+f}$, where FW_{fuel} is the formula weight of botryococcene.

2.2.4. Mass transfer limitations for gas transport and optimization

The *total* rate of consumption of each of the gases is the sum of the contributions from cell growth, cellular maintenance, and fuel production (Eq. (8)),

$$\left[\frac{dC_i}{dt}\right]_{\text{total}} = \left[\frac{dC_i}{dt}\right]_{\text{growth}} + \left[\frac{dC_i}{dt}\right]_{\text{maint}} + \left[\frac{dC_i}{dt}\right]_{\text{fuel}} \quad (8)$$

Based on this premise, the volumetric productivity of botryococcene is ultimately constrained by the mass transfer rate of the limiting gas substrate into liquid. At steady state conditions in the bioreactor, where liquid-phase gas concentrations are constant, the uptake rate of the i-th gas substrate by the organism $\left(\left[\frac{dC_i}{dt}\right]_{\text{total}}\right)$ is equal to the gas transfer rate of this gas component (GTR_i). For gaseous component i, these rates can be calculated from the gas phase fractional composition (y_i), the total gas pressure (P_{tot}), the gas–liquid mass transfer coefficient (k_La_i,), Henry's law coefficient (H_i) and the liquid phase concentration (C_i), as shown in Eq. (9):

$$\left[\frac{dC_i}{dt}\right]_{\text{total}} = GTR_i = k_La_i\left(\frac{y_iP_{\text{tot}}}{H_i} - C_i\right) \quad (9)$$

Thus, this equation illustrates that the GTR_i, and therefore the total possible gas uptake by the organisms, are limited by the k_La_i that is possible in a particular reactor type.

To simultaneously satisfy the organism's H_2, O_2, and CO_2 demands, there is only one set of y_i at which all the gases are

transported at a stoichiometrically balanced rate. At any other gas composition, only the limiting component will transport at the maximum rate ($[C_i] = 0$), while the other components are transported at a sub-maximal rate ($[C_i] > 0$). Therefore, the bioreactor must be operated at the gas composition that results in maximum transport rate of each of the gases to achieve the highest volumetric productivity. In practice, this could be achieved by controlling the gas phase composition via feedback control loops. In our simulations, we used the optimization functionality in Aspen plus® to converge on the optimal gas composition. Because the bioreactor k_La_i also affects the GTR_i, as described above, we constrained the optimization by limiting $k_La_{O_2}$ to values correlated for bioreactor configurations. Fixing $k_La_{O_2}$ then fixes $k_La_{H_2}$ and $k_La_{CO_2}$, as these are related to $k_La_{O_2}$ by the diffusion coefficient correction to mass transfer (Eq. (10)),

$$k_La_i = k_La_{O_2}\left(\frac{D_i}{D_{O_2}}\right)^{2/3} \quad (10)$$

An inter-conversion based on diffusion coefficient to 2/3 power is used. This is because correlations in literature are typically reported for $k_La_{O_2}$ as a function of operating conditions (rpm, gas rate etc.) and mass transfer coefficients have an inherent dependence on gas diffusion in the associated boundary layer, which needed to be captured in the analysis.

3. Results and discussion

Continuous operation provides for the high productivity required for fuel production. Extracellular botryococcene accumulation allows for the recycle of biomass for a higher operating concentration and higher process volumetric productivity. Since achieving economic feasibility is anticipated to be challenging, these operational enhancements are assumed as baseline requirements.

3.1. Sensitivity analysis: effect of reactor residence time (τ)

In a perfusion bioreactor, the residence time through the reactor system ($\tau = V/F$) and cell retention efficiency (ε) determine the required cell proliferation rate (μ) and operational cell concentration (X). These subsequently affect the rate at which fuel will be produced for a given specific fuel productivity (R_{fuel}). The economics of this process are highly dependent on the volumetric fuel productivity ($\bar{P}_f$; this sets the required reactor size and therefore the capital cost) and the fuel yield on H_2, (Y_{f/H_2}; the major operating cost). Therefore, we performed an initial set of simulations to evaluate the effect of reactor residence time on these two variables (Fig. 3). The steady state cell density in the reactor is also presented to assist in interpretation (Fig. 3C) and to establish the operational cell concentration that must be achievable using the host organism. For these simulations experimentally determined values for microbial energetic parameters were used (unpublished data): true growth yield ($Y^T_{H_2}$) and maintenance coefficient (m_{H_2}) of both *Rba. capsulatus* (2.66 gDW/mol-H_2 and 2.01×10^{-3} mol-H_2/gDW h, respectively) and *Rls. eutropha* (7.68 gDW/mol-H_2 and 6.8×10^{-3} mol-H_2/gDW h, respectively). Fig. 3 presents the results for the chosen process variables: $k_La_{O_2} = 330/h$, R_{fuel} = 0.5 g-fuel/gDW h and ε = 95%.

The results indicate that X, $\bar{P}_f$ and Y_{f/H_2} all increase with residence time, asymptotically approaching maximum values. This behavior results from the opposing effects of X and τ: in a continuous flow bioreactor, $\frac{1}{\tau}$ = growth rate of the bacteria (μ) = $\frac{1}{X}\frac{dX}{dt}$ (given $\frac{1}{\tau} < \mu_{max}$). Therefore, as τ increases, specific growth rate decreases. Since the volumetric rate of substrate availability is fixed by gas mass transfer (k_La), the reduction in specific growth rate and reduced gas consumption per cell permit an increased steady state cell concentration with an associated greater fuel production per unit volume. Based on the assumption that metabolism can be directed to produce fuel instead of cell mass results in operating at lower growth rates. At lower growth rates less of the available H_2 must be partitioned to cell growth, enabling more of the H_2 to be used towards synthesizing fuel, and enabling a higher Y_{f/H_2}. This is reflected in Fig. 3B.

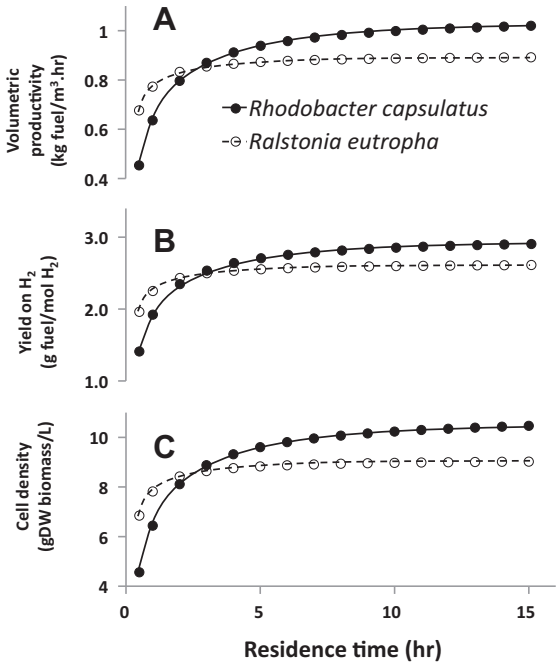

Fig. 3. Variation of volumetric productivity, R_{fuel} (A), fuel yield on H_2, Y_{f/H_2} (B) and cell density, X (C) as a function of residence time (τ) through the reactor for *Rhodobacter capsulatus* (●) and *Ralstonia eutropha* (o). Simulation parameters: true growth yield $\left(Y^T_{H_2}\right)$ of *Rba. capsulatus* = 2.66, *Rls. eutropha* = 7.68 gDW mol-H_2^{-1}, maintenance coefficients (m_{H_2}) of *Rba. capsulatus* = 2.01×10^{-3}, *Rls. eutropha* = 6.8×10^{-3} mol-H_2 gDW^{-1} h^{-1}, k_La = 330 h^{-1}, specific productivity = 0.5 g-fuel gDW^{-1} h^{-1}, cell recycle efficiency (ε) = 95%.

The preceding discussion also explains the important observation that at higher residence times (and therefore lower growth rates) the organism with lower maintenance requirement (*Rba. capsulatus*, in this case) is predicted to perform better despite its less efficient utilization of H_2 for growth. This is because at a lower growth rate, a smaller fraction of the available energy substrate is used for maintenance, leaving more H_2 available for fuel synthesis. The assumption that mass transfer limitations are the dominant constraint on bioreactor operation is central to this argument. Because it is desirable to operate at higher fuel yield and volumetric productivity, $Y^T_{H_2}$ and m_{H_2} of *Rba. capsulatus* and τ = 15 h were chosen for further simulations.

3.2. Sensitivity analysis: effect of specific productivity (R_{fuel})

Specific fuel productivity is an intrinsic biological property of a genetically engineered production strain, and affects the volumetric productivity as well as the fuel yield on H_2. The range of specific fuel productivities examined in this analysis spanned from 0.1 to 2 g-fuel gDW^{-1} h^{-1} (Fig. 4A). This is a wide range covering conservative to very optimistic specific productivity targets. As a reference, the ethanol specific productivities of *E. coli* growing on various fixed-carbon substrates reported in the literature range from about 0.3 to 0.8 g-EtOH gDW^{-1} h^{-1} (estimated from Ohta et al., 1991; Hildebrand et al., 2013). The resulting fuel yields on

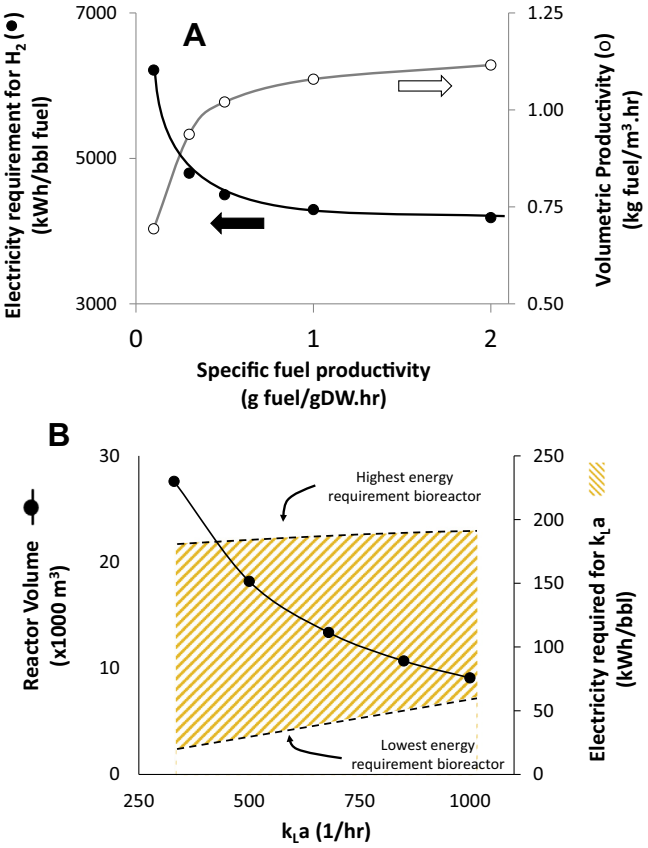

Fig. 4. (A) Variation of electricity requirement for H_2 generation (●) and volumetric productivity (o) with specific fuel productivity representing an increasing fraction of energy being converted into fuel instead of biomass (simulation parameters: residence time = 15 h; other parameters same as in Fig. 3). (B) Variation in reactor volume as a function of k_La showing the reduction in culture process volume that is enabled as the mass transfer rate is improved (●). The range of electricity requirements that would be required to achieve the specified k_La in different bioreactor configuration is shown as the shaded area between the dashed lines. The high and low value of the electricity requirement at each k_La corresponds to the highest and the lowest P/V able to produce the specified k_La; the range of P/V for a variety of bioreactor configurations are presented in Fig. 5.

H_2 were converted to electricity required (kWh/bbl-fuel), based on an average electrolytic efficiency of 80% for H_2 generation (Myers, 2013). This is the major component of the operating cost of the process. In the range of low specific productivities, the electricity requirement for H_2 generation initially decreases sharply as specific productivity increases. This is attributed to the fact that when specific productivity is low, the majority of the energy is expended in the growth of cells rather than ending up in the fuel molecule, so small improvements in specific productivity give large electricity cost savings. However, the electricity requirement remains essentially unchanged above specific productivities of 1 g-fuel gDW^{-1} h^{-1}. This asymptotic minimum is the sum of the energy fixed in the fuel molecule and the minimum loss that is associated with cell growth and maintenance.

Specific productivity is the target associated with genetic engineering and development of the host organism. Obviously, higher is better, but the more important question is: at what range of specific productivity will the process achieve economic viability? Initial efforts to introduce the botryococcene synthesis pathway via metabolic engineering approaches have successfully produced the botryococcene molecule (Khan et al., 2013); however, the observed production levels are more than an order of magnitude lower than the low-range values used in this simulation. For the current, experimentally observed productivity levels, it was calculated that the electricity cost would be about 175 times that of the 0.1 g-fuel gDW^{-1} h^{-1} specific productivity case. The genetic engineering efforts are well justified because a small increase in specific productivity (which is possible at the early stages of development) translate to large savings in electricity cost. However, after a significant productivity has been attained, and electricity requirements level off, other aspects of the process become increasingly important for achieving economic feasibility. For our subsequent analysis, the moderately optimistic level of 0.5 g-fuel gDW^{-1} h^{-1} was used.

3.3. Sensitivity analysis: effect of gas–liquid mass transfer coefficient (k_La)

The mass transfer rate affects the volumetric productivity and therefore determines the reactor volume required at a given production capacity, as discussed in Section 2.2.3. Increasing k_La comes at the expense of increased operating costs for mass transfer, but this facilitates a decrease in the capital cost due to a reduction in the required reactor volume. Fig. 4B shows the decrease in reactor volume and increase in energy cost (kWh/bbl-fuel) for an increase in k_La from 330 to 1000 h^{-1} for a range of bioreactor configurations. To serve as a guide in determining relevant reactor costs and realistic k_La values, the ranges of k_La used in Fig. 4B and the associated power-per-unit-volume (P/V) are based on values for various gas–liquid contacting reactors reported in the literature. The compilation is presented in Fig. 5. In performing the literature review, we focused on systems that can be operated at the large scale required for this process. The key is to choose a bioreactor where high mass transfer can be sustained at relatively low power levels. In this respect, trickle-bed reactors were found to perform the best at commercial scales. While microbubble reactors have similar performance, they have only been demonstrated at the laboratory scale. We have therefore used trickle-bed reactor costs in our final analysis.

Comparing the energy costs for k_La enhancements in Fig. 4B to the costs of energy for H_2 production in Fig. 4A, energy required for mass transfer is only 1.5–4.5% of the minimum energy required for

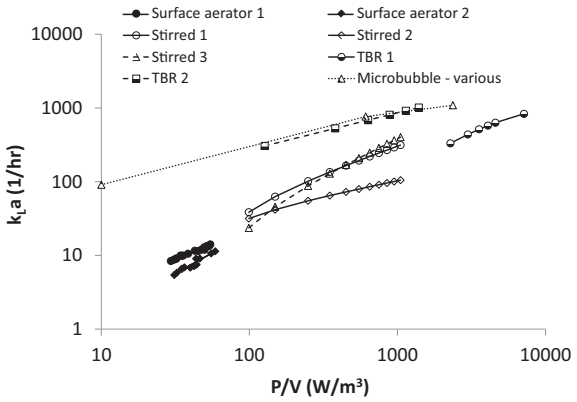

Fig. 5. Gas–liquid mass transfer coefficient (k_La) vs. power/volume (P/V) for various reactor types. Surface aerator 1 = wastewater treatment plant demonstration run, Chattanooga, TN (Cosby & Gay, 2003); surface aerator 2 = wastewater treatment plant demonstration run, Yuba City, CA (Lewis & Gay, 2003); Stirred 1 = stirred tank reactors correlation for electrolytes, (Van't Riet, 1979), V_s = 0.005 m/s; Stirred 2 = stirred tank reactor correlation for non-electrolytes (Van't Riet, 1979), V_s = 0.005 m/s; Stirred 3 = stirred tank reactors correlation for electrolytes (Linek et al., 1987), V_s = 0.005 m/s; TBR 1 = trickle-bed reactor correlation set 1 (Reactor parameters: Roininen et al., 2009, k_La correlation: Goto & Smith, 1975, pressure-drop correlation: Larachi et al., 1991); TBR 2 = trickle-bed reactor correlation set 1 (reactor parameters: Roininen et al., 2009, k_La correlation and pressure-drop correlation: Reiss, 1967); Microbubble-various = experimental data from various investigators (Bredwell & Worden, 1998; Hensirisak et al., 2002). This compilation of literature values focuses on large-scale commercial units (except for microbubble technology).

H_2 generation (~4000 kWh/bbl-fuel). This result emphasizes the dominance of H_2 generation requirements in overall energy requirements. Optimizing k_La, therefore, will only become important in the later stages of plant design when more accurate equipment costs are available. Therefore in determining the total capital and operating cost of the process, we selected a k_La of 1000 h^{-1}.

3.4. Process economic analysis of capital and operating costs

The preceding analysis, based on specific productivity and mass-transfer requirements, helps define the realistic range to conduct a more detailed analysis of capital and operating costs. Table 1 shows the values selected for parameters used in this section of the work, all of which are based on the calculations described above. The scaled-up process design is based on a daily production rate of 5000 barrels of oil per day (795,000 L/d). As a reference point, in 2012 the largest ethanol production facility in the United States had a nameplate capacity of slightly over 27,000 bpd, while the average facility produced around 4500 bpd (Nebraska, 2014).

A capital cost analysis provides insight into the overall economics of a production platform by (1) identifying the relative costs of the various components and (2) quantifying the relative magnitude of the capital cost in relation to the fixed and operating costs. The capital costs of the production equipment were determined using Aspen plus® software, except for electrolysis cells, whose capital costs were calculated based on literature data (Saur, 2008). We did not include the capital costs of the renewable electricity generation component in our analysis because this is accounted for through the use of Levelized Cost of Electricity (LCOE) for a particular electricity source; therefore, these costs are incorporated into the cost of H_2. This choice was made because reliable and up-to-date studies on the costs of various renewable electricity technologies already exist (IRENA, 2012; REN21, 2013). The results of the capital cost analysis are presented in Fig. 6A; as can be seen, the costs of the process components varied over a range of three orders of magnitude. We grouped the equipment into categories of lower and higher cost, and note that the capital cost of photovoltaics included in H_2 generation costs corresponds to the highest capital expense.

The capital cost of the platform (excluding electricity production) is $24.44/bbl-fuel, assuming an after-tax rate of return of 15% (Fig. 6B). The fixed cost component was much smaller than the capital cost component at $8.81/bbl-fuel. The operating cost of the process is highly dependent on the cost of electricity and the LCOE of electricity is quite variable for the various types of renewable energy technologies. However, the cost of nutrient feed-water, wastewater, cooling water, and CO_2 are relatively insensitive to electricity cost, and amount to ~$18.9/bbl-fuel. Although we expected CO_2 cost to be minimal due to it being a waste product, surprisingly, this cost was found to be the most significant cost after electricity. In this analysis we assume that CO_2 from a power plant or a similar point source will be purified, compressed, and transported to this facility for use. The magnitude of this cost varies substantially with the types of point source, carbon capture technology employed, and the means of transport. The average of the cost of capture and compression of CO_2 for IGCC, NGCC and PC power plants is reported to be $31/tonne-$CO_2$ (Metz et al., 2005). Transportation costs for CO_2 via pipeline or tanker transport varies from 1 to 5 USD/tonne-CO_2/250 km (Metz et al., 2005). Using an average cost of $3/tonne-$CO_2$/250 km and an average distance of 500 km between the CO_2 point source and the Electrofuels plant, we predict CO_2 transportation costs of $6/tonne-$CO_2$. Thus, using the total predicted CO_2 cost of $37/tonne-$CO_2$ results in a platform cost of $17/bbl-fuel. As shown in Fig. 6B, the overall cost of the Electrofuels production platform (excluding the cost of H_2 and the much smaller bioreactor operational requirements for mass transfer) is therefore estimated to be around $54.3/bbl-fuel, a price well below the target sales price of $127/bbl-fuel (based on the DOE's estimate of the 2020 price of oil (Gruenspecht, 2012)).

The challenge is therefore to determine the true effect of renewable electricity costs on this technology, which is complicated by the range of various technologies available and the large variability of their costs in North America and around the world. The International Renewable Energy Report (IRENA, 2012) shows that the worldwide LCOE of various renewable energy technologies varies from as low of 4 ¢/kWh for onshore wind power to a high of >30 ¢/kWh for large-array solar PV. For North America the averages range from 4 ¢/kWh for hydropower to 50 ¢/kWh for solar PV (IRENA, 2012; REN21, 2013). The range of final fuel costs, based on the potential range of renewable electricity costs, is shown in Fig. 6C. As can be seen, the range of final fuel costs spans a very wide range, which reflects the wide variation found in the renewable electricity sources. Furthermore, the lowest projected cost (187.2–748.8/bbl-fuel; based on onshore wind electricity) is still about two to six times higher than the 2020 crude oil price estimate ($127/bbl-fuel) (Gruenspecht, 2012). The highest costs are generally for solar photovoltaics, which are about twenty times that of this baseline crude oil price.

To place the cost of these electricity generation technologies in perspective, we are presenting a brief comparison based on two traditional, fossil fuel based technologies for H_2 generation: coal gasification and natural gas reforming. In a recent study, (Bartels et al., 2010) reported hydrogen production costs of 0.36–1.83 $/kg and 2.48–3.17 $/kg from coal and natural gas respectively. This produces ranges of 68.8–205.4 and 265.8–330 $/bbl-fuel respectively based on the Electrofuels scenario. Although much lower in cost than some of the renewable technologies for H_2 generation, both coal gasification and natural gas reforming generate a much higher ratio of CO_2 to H_2 than can be consumed by the process. From our analysis, we found that the maximum ratio of consumption of CO_2 to H_2 in the Electrofuels process is 0.2 mol-CO_2/mol-H_2. On the other hand, the best of coal gasification and natural gas reforming technologies would generate 1.08 and 0.42 mol-CO_2/mol-H_2. According to these numbers, at least 81% and 52% of the CO_2 generated by these technologies respectively would be either released to the atmosphere or have to captured by some other process, making them environmentally unsustainable.

It is obvious that at the current crude oil prices and renewable electricity costs, the Electrofuels process is not economically favorable. There are several opportunities that were not taken into account in this analysis that could provide for somewhat improved economics. These include cost reductions for carbon credit, use of low-cost plastic fermentation systems (Hsiao et al., 1999), use of the waste biomass from the process for heating, etc. Furthermore, some of the LCOE costs used in the analysis include capital and operating costs for transmission and distribution of the electricity to the grid, which is not necessary if a direct electrolysis process is adopted. Nonetheless, the overall analysis provides quantitative evidence that even with improvements and meeting cellular productivity metrics, the proposed process does not achieve economic feasibility in terms of producing a fuel product. It is not entirely

Table 1
Parameter values used in final scaled-up design.

Parameter	Value
Plant size	5000 bbl/day
Specific productivity	0.5 g-fuel gDW^{-1} h^{-1}
Oxygen mass-transfer coefficient (k_La)	1000 h^{-1}
Cell recycle (ε)	95%

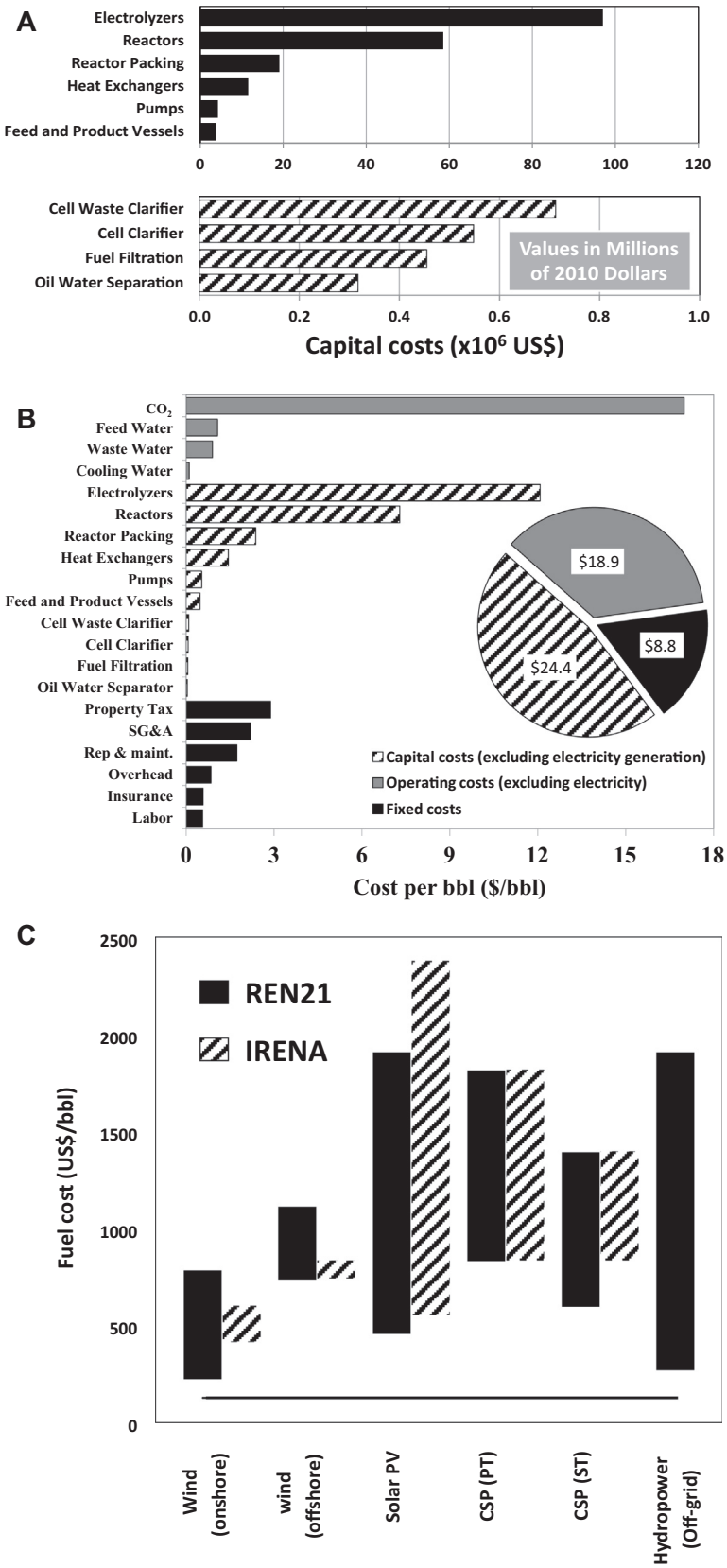

Fig. 6. (A) Capital costs of various process components for a 5000 bbl/day fuel production plant. Results are grouped into high (black bars) and low (dashed bars) range capital costs. (B) A breakdown of process costs (the dominant cost of electricity generation is not presented). Top (gray) group represents the major operating costs which make up 36% of total; middle (hashed) group represents the major non-photovoltaic capital costs which make up 46% of total; bottom (black) group estimates the major non-PV fixed costs corresponding to 17% of the total. (C) A sensitivity analysis of the final fuel cost based on LCOE of various generation methods reported in two different sources: REN21, 2013 (black); IRENA, 2012 (dashed). CSP = Concentrate Solar Power, PT = Parabolic Trough, ST = Solar Tower. The horizontal line indicates year 2020 crude oil price estimate (Gruenspecht, 2012). Off-grid hydropower values were not available in IRENA, 2012.

unexpected that an electrofuel would have difficulty competing with crude oil at the current price, as the energy content of crude oil is a result of millions of years of accumulation.

3.5. Alternative perspective

A potential advantage of the hypothetical electrofuels process that is not captured in this analysis is the potential for using this microbial platform to make alternative, high-value products by leveraging the high specificity of synthesis that is an inherent capability of biological systems. Through the use of genetic engineering, the autotrophic host could be designed to produce biochemical products with high degree of specificity. For example, in parallel to the genetic engineering efforts to produce botryococcene, we have also demonstrated squalene production in *Rba. capsulatus* (unpublished data). The current bulk price of squalene is $15–20/kg, which translates to about $2027–2702/bbl-fuel. Other isoprenoids, such as lycopene, valencene, beta-carotene, etc., are also high value products whose production are demonstrated in non-native hosts (Alper et al., 2005; Beekwilder et al., 2014; Verwaal et al., 2007). Wax ester (a health product commonly sold as Jojoba oil) is a fatty ester for which we have also demonstrated production in *Rba. capsulatus* (unpublished data). At ~$68/L, the retail price of Jojoba oil translates to a price of $8064/bbl-fuel. These examples demonstrate the potential value of alternative products able to be generated by a biological production platform using a sustainable, renewable substrate. This alternative paradigm for Electrofuels commercialization could facilitate the simultaneous production of multiple high value products (different reactors employing different genetically modified strains) using remote sources of electricity/energy. This could be an alternative use of the electrofuels process during the technology development phase until reductions in the cost of renewable electricity and/or the rise in the price of crude oil make Electrofuels production economically feasible.

3.6. Targets based on the analysis

One of the primary goals of this analysis was to first establish the effect of the key process parameters – $Y^T_{H_2}$ and m_{H_2} of the bacteria, τ, R_{fuel} and k_La – on the cost of fuel produced by an Electrofuels process, and subsequently define the target productivity metrics for potential feasibility. The range of values for R_{fuel} and k_La used in this analysis were selected based on realistic engineering considerations of what is actually feasible, while $Y^T_{H_2}$ and m_{H_2} are measured physiological values. The value of the residence time parameter (τ) is subsequently constrained by these parameters.

It was found that the cost of H_2 generation (i.e. the cost of electricity) accounts for >90% of the current cost of fuel from the Electrofuels process. The next largest costs are the capital investment required for other non-H_2 process equipment and the operating costs for providing mass transfer and supplying CO_2. Therefore, steps that decrease H_2 consumption would drastically improve process economics. Critical steps include using a host organism with high $Y^T_{H_2}$ and low m_{H_2}, and improving the specific fuel productivity to achieve a target >0.3 g-fuel gDW^{-1} h^{-1}. The sharpest change in H_2 cost occurs at specific productivities below 0.5, where H_2 costs escalate rapidly. This means that incremental changes in productivity in this range have a large impact on the process economics. Also, since the cost of k_La is low relative to the H_2 generation cost, and because increases in mass transfer have the potential to drastically decrease the required reactor volume (and thus reduce capital cost), it is advantageous to use a bioreactor that can achieve large k_La, even at the expense of increased energy costs for achieving a target k_La.

The other major goal of this analysis was to assess the overall economic feasibility of an Electrofuels process. It was found that above a specific fuel productivity of 0.3, the process can be feasible at an LCOE < 2¢/kWh at the current crude oil prices; this LCOE is about 2–20 times lower than the cost of electricity by current renewable electricity technologies. Given that the economic feasibility is the primary concern, producing fuels via the Electrofuels process is not a viable option at the moment. However, the various technologies considered in this analysis (renewable electricity, autotrophic host, engineering and energetics of hydrocarbon pathways) are in early developmental phase and have the potential to drastically change the economics in future. Alternative higher-value biochemical targets such as lubricants may be pursued during this phase of development to act as economic motivator for near-term profitability, so that the technologies can be developed for application when the rising crude oil prices (due to the depletion of reserves) allow acceptable economics for this process.

4. Conclusions

Cost of electricity (for H_2 generation) was found to have the largest impact on process economics. Given the assumptions made and current crude oil prices, the process is expected to be feasible at an LCOE $\leqslant$ 2¢/kWh. Performance parameters examined in this analysis included both organism-specific physiological parameters, and parameters for process operational modes. A critical requirement for economic feasibility is an autotrophic host with a high yield on hydrogen, low metabolic maintenance energy requirements, and able to meet a specific fuel productivity metric of >0.3 g-fuel gDW^{-1} h^{-1}. Gas–liquid mass transfer capabilities of the bioreactor were not found to be critical.

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Appendix A. Supplementary data

Additional information and clarification can be obtained from the supplementary material for Sections 2.2 and 2.2.1. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2014.08.118.

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