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Proposed design of distributed macroalgal biorefineries: thermodynamics, bioconversion technology, and sustainability implications for developing economies

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Abstract: Biomass to fuel programs are under research and development worldwide. The largest biomass programs are underway in industrialized countries. In the coming decades, however, developing countries will be responsible for the major increase in transportation fuel demand. Although the lack of existing large-scale infrastructure and primary resources preclude oil refining in developing countries, this provides an opportunity for the rapid implementation of small-scale distributed biorefineries to serve multiple communities locally. The principles for biorefinery design, however, are still in their infancy. This review sets a precedent in combining thermodynamic, metabolic, and sustainability analyses for biorefinery design. We exemplify this approach through the design and optimization of a marine biorefinery for an average town in rural India. In this combined model, we include sustainability and legislation factors, intensive macro algae *Ulva* farming, and metabolic modeling of the biological two-step conversion of *Ulva* feedstock by a yeast (*Saccharomyces cerevisiae*), and then by a bacterium (*Escherichia coli*), into bioethanol. We hope that the model presented here will be useful in

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considering practical aspects of biorefinery design. © 2013 Society of Chemical Industry and John Wiley & Sons, Ltd

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Key words: biorefinery design; thermodynamic modeling; biorefinery optimization; fermentation modeling; metabolic modeling; biofuel sustainability; biofuel policy

Introduction

The economically efficient and socially and environmentally sustainable conversion of solar energy into valuable products is a major contemporary challenge for governments and businesses worldwide. Transportation fuels, electricity, heating, cooling, drinking water, food, animal feed, chemicals, and materials are all potential products of solar energy conversion. One of the pathways to convert solar energy into useful products is biorefining. Biorefinery systems integrate the capture of solar energy and carbon dioxide (via plant photosynthesis), biomass harvesting and processing, and distribution of bioenergy and other biomass-derived products. The process of designing a biorefinery is a complex task and is largely influenced by local raw material supplies and socio-economic conditions.

The United States Department of Energy (DOE) recently published a multi-year plan for the strategic development of biomass sources.[1] This plan includes a large-scale (≥20 million gallons per year[2]), integrated network of biorefineries, as shown in Fig. 1(a).[3] In this plan, multiple

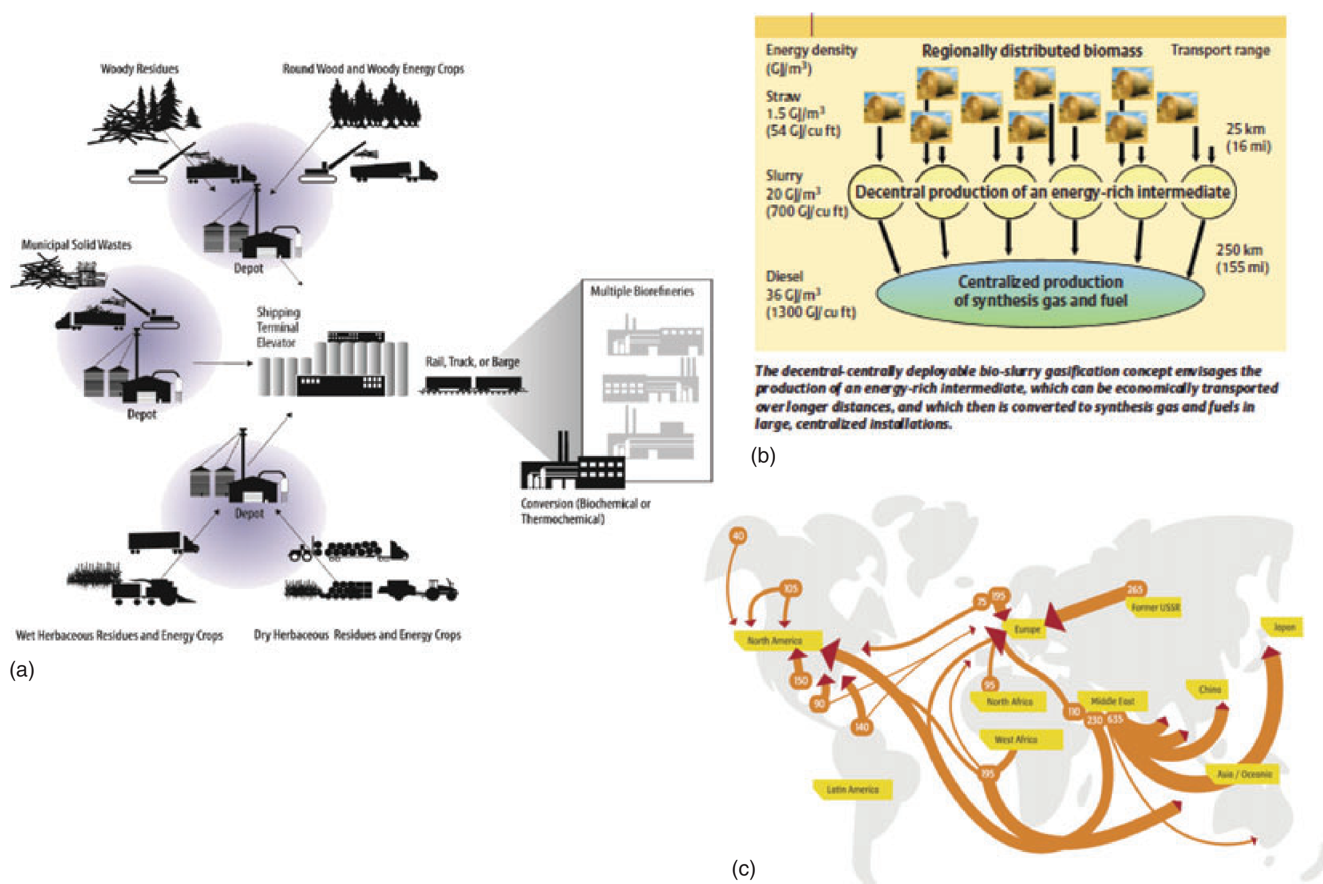

Figure 1. Large-scale biorefinery and global oil transportation systems. (a) US DOE Advanced Feedstock Processing Supply System. Figure courtesy of Idaho National Laboratory.[3] (b) KIT Bioliq® process.[3] © Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission. (c) Global crude oil transportation routes.[5] Source Cedre, designed based on Comité Professionnel Du Pétrole, *Pétrole 2004 : éléments statistiques* – Design Hippocampe. Reproduced with permission.

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local biomass units harvest and process raw biomass, and ship biofuel intermediates to large-scale biorefineries for subsequent fuel conversion, separation, processing, and distribution. A similar program, Bioliq®, under development in Germany, is shown in Fig. 1(b).[4] The cost of transporting biomass to a biorefinery provides an external limit to the size of the biorefinery. Increasing biomass energy density to make transportation more cost-effective is one strategy pursued by biorefinery programs to address this limitation. The approach of large-scale, integrated, biofuel supply chains may provide solutions for industrialized countries with large urban populations, but their implementation will likely take tens of years, and uncertainty remains as to their overall sustainability. Adapting the large-scale biorefinery concepts, described in Figs 1(a) and 1(b) to a global scale may result in the worldwide transport of biofuels or biofuel intermediates, as has already been shown,[5] whereby countries with abundant biomass resources provide raw materials for large-scale biorefineries in a few global locations. Extrapolating new biomass logistics systems to the global level could lead to a system resembling current global oil transport and processing where: (i) large deposits of oil are explored at few global locations; (ii) oil is transported via a small number of main routes to large-scale refineries (Fig. 1(c));[6] and (iii) refined products are distributed through local distribution chains. To avoid this, a global biofuel supply chain will need to prove social, economic, and environmental sustainability.

Although the key biofuel programs are under development in industrialized countries,[7] the major growth in liquid fuel demand over the next 20 years will be predominantly due to rising consumption in developing countries.[7,8] In Organization for Economic Co-operation and Development (OECD) countries, gross domestic product (GDP) is not linearly correlated to primary energy production; however, in developing countries GDP growth requires an increase in primary energy production.[8,9] The World Energy Council predicts that India and China will overtake developed countries in transportation fuel consumption by 2025.[10] Therefore, the largest need for new refinery infrastructure is in developing countries.

Our work focuses on developing methods to design adaptable biorefineries in order to meet transportation fuel demands on a small, local scale. This approach will enable multiple rural communities in developing countries, such as India, China, and countries in Africa, to generate and process biomass in order to supply local transportation fuel demands without relying on foreign production and refining. Recently, we introduced the concept of distributed marine macro-algae-based biorefineries.[11] We proposed the use of distributed networks of marine biorefineries to supply transportation fuel needs to the rural population. The goal of this work is to provide a high-level modeling methodology for the design of biorefineries to meet local needs for transportation energy in developing countries. We target these populations since the majority of new biofuel systems to be built in future years will serve their growing demands.[10] We report on the combined thermodynamic, technological, and policy development aspects to design marine biorefineries that will meet the needs for transportation fuel in developing countries. First, we provide the governing equations to optimize the biorefinery size and the serviced area. Second, using a two-step yeast then bacteria fermentation model, we show how metabolic models of feedstock bioconversion into liquid fuels are integrated into overall biorefinery design. Third, we discuss the sustainability and policy principles for the marine biorefinery design. Our work implies that a combined analysis of biorefinery components is necessary to accelerate the sustainable implementation of biorefineries in rural communities of developing countries, enhancing local capacity to meet the growing local need for transportation fuel.

Methodology

The goal of this section is to develop a set of governing equations for modeling and optimizing biorefinery scale and service area size. We develop a set of equations for a single biorefinery for a given population density and per capita transportation fuel consumption rate. In the following section we describe the implementation of a suggested methodology for specific examples of macroalgal biorefinery design.

Thermodynamic model for biorefinery scale optimization

Our approach is inspired by Constructal design which demonstrates how the optimal distribution of products and services flows across a populated area depends on a balance between the size of the production centers and the sizes of distribution networks that connect these centers to end users.[12–15] Although larger systems are thermodynamically more efficient in production, they serve larger areas; thus, the collection and distribution logistical costs also increase with size,[15–17] as do potential environmental impacts and public opposition.[18] Therefore, a balance exists between efficiencies of scale, distribution system losses, environmental impact, and public acceptance. This

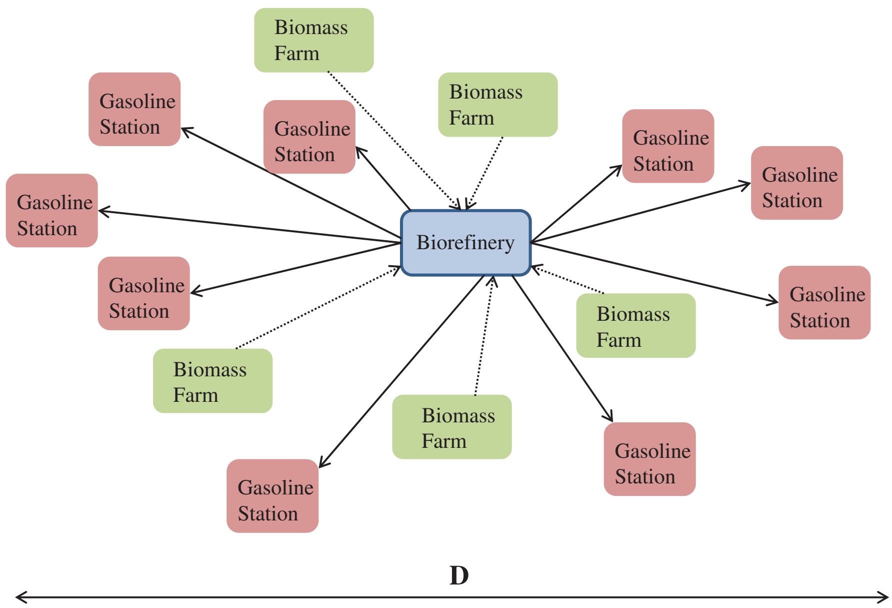

Figure 2. Scheme of a single biorefinery service area with characteristic length *D*. Feedstock is supplied by multiple local producers concentrated within an area of characteristic length scale *nD* around the biorefinery. The produced liquid fuel is consumed within the service area.

balance has been used for analyzing both the thermodynamics of energy sources[13–15] and the economics of agricultural/biofuel systems.[19–21] Applications of the balance principle have been demonstrated for various systems such as the thermodynamic optimization of hot water flow and heating,[13] refrigeration,[14] combined solar power and desalination,[15] and agricultural product processing economics.[19] The balance principle has also led to proposals for distributed energy systems to optimize energy production size and service area.[15] In this work we add biorefineries to the distributed energy network (Fig. 2).

Each biorefinery collects feedstock biomass from the surrounding farming area of characteristic length scale nD, processes it to liquid biofuel, and then distributes the biofuel to the population at specific locations within the service area. The scheme of the local biorefinery serving a network of biomass producers and liquid biofuel consumers is depicted in Fig. 2. The service area is parameterized by characteristic length D, population size N, population density ρ, and per capita transportation fuel consumption rate m_1. The area D^2, through which the population is homogeneously spread, provides space for food and fuel crops, and solar energy systems. The rate of biorefinery transportation biofuel production that exactly meets the population's demand is given by:

$$m = m_1 N = m_1 \rho D^2 \qquad (1)$$

Figure 3 depicts the thermodynamic model of the biorefinery service area shown in Fig. 2. Fuel crops (residing in a fractional allocated area $(nD)^2$) collect solar flux Q_{solar}. Photosynthesis, the conversion of solar energy to chemical energy stored in biomass, has limited efficiency (4–6%).[22] W_0 is the photosynthesis solar energy loss. The resulting fuel crop biomass is harvested and transported to the biorefinery, requiring a truck, train or a boat, which consumes a certain amount of fuel per km of transport.

The feedstock collection energy loss W_1 is related to transport length L_t by:

$$W_1 = c_1 L_t = N m_1 \qquad (2)$$

where c_1 is the transportation vehicle efficiency coefficient. French[19] has shown that the total transportation length is proportional to the characteristic length of the area:

$$L_t = c_2 nD \qquad (3)$$

where c_2 is a proportion coefficient.

Once delivered to the biorefinery, the fuel crops are converted to liquid fuels. The conversion efficiency depends on multiple factors such as crop type and processing technology. Previous analyses of biorefineries[23] and other power conversion systems[17,24] suggest that conversion efficiency increases with biorefinery size. W_2 is the energy lost during the crop to fuel conversion process:

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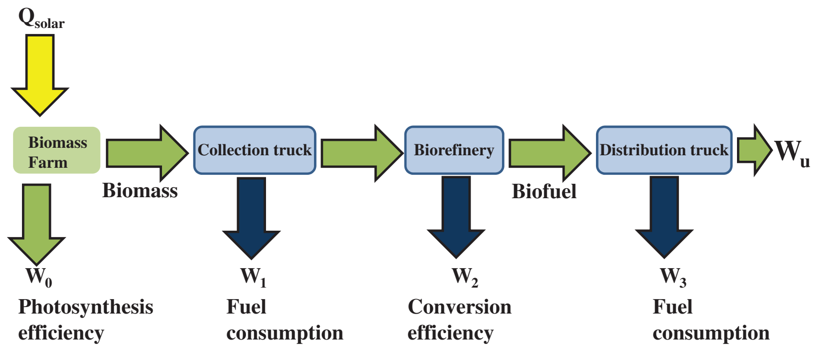

Figure 3. Thermodynamic model of a biorefinery producing liquid transportation biofuel. Q_{solar} is the solar energy flux, W_0 is the photosynthesis energy loss, W_1 is the biomass collection energy loss, W_2 is the fuel conversion energy loss, W_3 is the fuel distribution energy loss, and W_u is the total useable transportation fuel energy supplied to the population.

$$W_2 = c_3 m^{\alpha} \tag{4}$$

where α is a scale factor ($\alpha < 1$) and c_3 is a proportion coefficient.

Finally, the produced biofuel is distributed to consumers (Fig. 3). The biofuel distribution energy loss is given by:

$$W_3 = c_1 L_d N m_1 \tag{5}$$

where the length of distribution roads (L_d) depends on the service area characteristic length as follows:[19]

$$L_d = c_4 D \tag{6}$$

where c_4 is a proportion coefficient.

The following equation describes the overall energy balance for conversion of solar energy to transportation fuel in our model:

$$W_u = Q_{solar}(nD)^2 - W_0 - W_1 - W_2 - W_3 \tag{7}$$

In order to maximize the total useful energy (W_u), it is necessary to minimize the energy losses. We assume that the photosynthetic energy losses are location and bioenergy crop specific. A review of photosynthetic efficiency optimization can be found in Zhu *et al.*[22] Here, we seek to minimize W, the aggregate feedstock collection, conversion, and fuel distribution energetic losses:

$$W = W_1 + W_2 + W_3 \tag{8}$$

or:

$$W = c_1 L_t N m_1 + c_3 m^{\alpha} + c_1 L_d N m_1 \tag{9}$$

Equations (1) to (9) lead to the total energy loss per consumer for producing and distributing biofuel:

$$WN^{-1} = c_1 c_2 n D m_1 + c_3 D^{2\alpha-2} m_1{}^{\alpha} \rho^{\alpha-1} + c_1 c_4 D m_1 \tag{10}$$

With increasing service area characteristic length D, the first and the third terms of Eqn (10) increase, while the second term decreases. In order to find the optimal service area leading to minimal energy loss per consumer, we differentiate WN^{-1} with respect to D and solve for D_{opt}, zeroing the derivative:

$$D_{opt} = \left\{ \frac{c_1 c_2 n + c_1 c_4}{(2-2\alpha) c_3} (m_1 \rho)^{1-\alpha} \right\}^{\frac{1}{2\alpha-3}} \tag{11}$$

or:

$$D_{opt} = (m_1 \rho)^{\frac{1-\alpha}{3-2\alpha}} \left\{ \frac{c_1 c_2 n + c_1 c_4}{(2-2\alpha) c_3} \right\}^{\frac{1}{2\alpha-3}} \tag{12}$$

The scale factor α was found to be approximately 0.7 for biorefineries.[25] Thus for D_{opt} and m_{opt}, we have:

$$D_{opt} = 0.73 \left(\frac{c_1}{c_3}\right)^{-0.63} (m_1 \rho)^{-0.19} (c_2 n + c_4)^{-0.63} \tag{13}$$

$$m_{opt} = m_1 \rho D_{opt}^2 \tag{14}$$

or:

$$m_{opt} = 0.53 \left(\frac{c_1}{c_3}\right)^{-1.26} (m_1 \rho)^{0.62} (c_2 n + c_4)^{-1.26} \tag{15}$$

The maximum efficiency of a local biorefinery is:

$$\eta_{\max} = 1 - \frac{W_{\min}}{Q_{solar}(n D_{opt})^2} \tag{16}$$

where $W_{\min}$ is the minimum wasted energy. $W_{\min}$ is obtained when the biorefinery serves the area $D_{opt.}$ Therefore,

$$\eta_{\max} = 1 - \frac{W_0 + c_1 c_2 n m_1 N D_{opt} + c_3 (m_1 \rho D_{opt}^2)^{\alpha} + c_1 c_4 m_1 N D_{opt}}{Q_{solar}(n D_{opt})^2} \tag{17}$$

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From Eqns (14) and (15) we see that the optimal service area decreases with increasing population density and per capita fuel consumption rate. In addition, the optimal biorefinery size grows as does $m_1^{0.62}$ for the per capita fuel consumption rate and as $\rho^{0.62}$ for the population density. Thus, as population density and per capita fuel consumption increase, we anticipate that biorefinery density and size will also increase.

In summary, we find that the optimal service area of a biorefinery decreases with increasing population density and per capita fuel consumption. This finding is in agreement with previously reported economic analyses.[19,21]

Crop sustainability index (CSI)

As soon as biofuel crops began to play a significant economic role, concerns were raised over their possible competition with food crops for arable land and potable water (and thus their potential threat to global food prices), as well as their potential adverse impact on the environment due to increased fertilizer and pesticide use (26). Following triple-P (profit, planet, and people) bottom line sustainability accounting principles, we propose sustainability metric for a biofuel feedstock crop as follows:

$$CSI = m^{-1}(s_1 W_0^{-1} + s_2 W_2^{-1} + s_3 PW^{-1} + s_4 Ch^{-1} + s_5 J) \qquad (18)$$

where *CSI* is the crop sustainability index, *PW* is the potable water used for irrigation of biomass processed by a single biorefinery, *Ch* are the accumulative tonnage of chemicals introduced to the environment during biomass cultivation, and *J* is the number of new jobs created. Each society may differentially weigh (s_1–s_5) the separate factors contributing to the *CSI* according to its unique set of circumstances important for local population.

As biofuel capacity increases, the choice and source of raw material will become increasingly important. The distribution of local resources such as land and water will prescribe the type of crops used for biofuels. In this scenario, metrics such as *CSI* will contribute to crop choice decision making.

Macro algae as an energy crop

The proper choice of raw biomass material is critical to ensuring the efficient production of transportation biofuels. First-generation feedstocks include sugar cane, sugar beets, starch-bearing grains, and conventional vegetable oil crops; while first-generation fuel products include ethanol and biodiesel.[7] Second- and third-generation biofuel technologies, currently in research and development, utilize animal fat, lignocellulosic biomass, and algae feedstocks; and produce hydrotreated vegetable oil, cellulosic-ethanol, biomass-to-liquids (BtL)-diesel, bio-butanol, and advanced drop-in replacement fuels such as fatty-acid ethyl esters, alkanes, alkenes, terpenes, and methyl ketones.[7,27–31] A large portfolio of thermochemical and biological conversion technologies is under development in large-scale biomass programs. Despite the promise of biofuel to satisfy a significant portion of the future demand for transportation fuel, the perceived competition between ‘energy crops’ and ‘food crops’ for land and water resources is a growing concern.[7,32] Furthermore, the extent to which land erosion, potable water consumption, fertilizers, pesticides, biodiversity and climate change impact biofuel sustainability have yet to be evaluated.[33]

Concerns over net energy balance, land and potable water use, and environmental hazards, question the sustainability of a corn/sugar cane biofuel future.[7,24,25,32–35] While lignocellulosic feedstock alternatives do not directly compete with the food supply, they may not be able to fully address land and potable water use, and environmental hazard concerns. In many locations around the world, arable land and potable water are scarce, but fuel demand exists. Furthermore, the technology required to release and ferment sugars from lignocellulose is still challenging.[2,36–38]

Macro algae, which contain very little lignin and do not compete with food crops for arable land or potable water, are potential additional candidates for future transportation fuel feedstocks.[28,39–42] Both developed and developing countries have recently reported efficient conversion of macro algae to transportation fuels.[28,43] Various green, red, and brown macro algae species are under evaluation for inland and offshore cultivation.[28,39,44] The macro algae from *Ulva sp.* is of particular interest due to fast growth rates[41,45] and high carbohydrate content. Yantovski[45] has proposed land grown *Ulva* for the production of 100% of electricity supply in Israel. Nevertheless, technological immaturity at large-scale (~100 km^2 of cultivation are required per GW of power) limit the immediate implementation of this program. As we show in this paper, however, with the currently available level of technology it is possible to design and build smaller scale systems for developing economies.

Intensive *Ulva sp.* farming

There are three major approaches to macro algae biomass production. The first is the direct harvest of drifting macro algae from ocean shores. Although the production cost of this approach is low, material availability and composition

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vary with climate, season, local agricultural effluents, etc., making it unsuitable for continuous fuel production. Extensive and intensive macro algae farming,[46] constitute the second and third approaches. Though extensive macro algae farming does not require mechanical mixing or active fertilization, it yields 3–4 times less than intensive cultivation.[47,48] Intensive *Ulva* farming systems have been previously reported.[41,49] The basic system includes: farming ponds, energy, fertilizers, and CO_2 supply for biomass cultivation and refineries for conversion into biofuel.

Achieving intensified biomass yields in on-shore macro algae cultivation ponds requires additional electrical power for mechanical mixing during active photosynthesis.[48] Since this additional power is only required during the day, we propose solar photo-voltaic (PV) systems to generate the required energy, thus integrating solar electrical power generation with intensive macro algae cultivation. Based on previous analyses of distributed desalination systems,[15] we suggested a central solar power station to supply electricity to the network of local cultivation facilities and refineries.[11] The primary analysis of the infrastructure, PV system size, fertilizers demand and CO_2 demand for intensive macro algae cultivation appears in our previous work.[11] Thus, modular biorefineries will be integrated into distributed energy networks, with electricity supplied from a large central facility, as proposed by Lorente *et al.*,[15] and biofuels will be produced locally at each biorefinery.[11] An additional assumption made in this model is that it is possible to use waste CO_2 from local industry. Combining CO_2 mitigation with macro algae cultivation has been reported previously.[50] Fertilization can be achieved through direct fertilizer input, circulating combined aquaculture systems,[49,51] or by circulating fresh sea water with natural nutrients. Nevertheless, nutrients lost to mixing between the cultivation ponds and open coastal waters could negatively affect both economics and sustainability.

Although intensive cultivation demands mixing energy and fertilization, the system leads to controllable, year-round production of biomass with constant chemical composition. The macro algae cultivation system proposed here consists of multiple ponds with mixing wheels and fertilization mechanisms. Due to its efficiency, the relatively low-tech system can be manufactured onsite in developing countries without sophisticated equipment.

Biological conversion of *Ulva lactuca* into biofuel

In this section, we analyze several scenarios for bioconversion of *Ulva lactuca* into ethanol using computational metabolic modeling.

Challenges and possibilities of *Ulva* bioconversion

Until recently, macroalgal biofuels have not been deployed as lignocelluloses feedstocks, despite potential advantages in reduced land and sea water use, and in comparison with micro algae, lower energy inputs for cultivation and harvesting. Limitations of macroalgal biofuels feedstock are the availability of enzymes to decompose specific macro algae polysaccharides (such as ulvan), as well as the lack of tractable microorganisms that can efficiently convert the resulting mono-saccharides, mainly xylose and rhamnose, into biofuels. Nevertheless, as evident from recent reports,[52–54] industrial research institutions and enzyme producing companies have started to pursue macro algae conversion efforts.

Xylose and rhamnose, resulting from hemicellulose and ulvan decomposition, often comprise up to 29% of biomass.[55] The yeast *S. cerevisiae*, one of the most promising organisms for biomass-derived biofuel production, poorly utilizes these sugars. Due to high carbohydrate content, theoretically up to 60% of the dry biomass in *Ulva sp.* could be fermented into ethanol.[56] However, current ethanol yields for *S. cerevisiae Ulva* bioconversion are reported at only ~14% of biomass dry weight (dw).[56] To make macro algae *Ulva* competitive with lignocellulosic ethanol production, average yields should be increased to 20–25%.[57] The multiple economic, cultivation, and environmental advantages of *Ulva* feedstock (listed above) provided improvements in biomass processing technology, would make *Ulva sp.* a preferable biofuel feedstock, especially in coastal regions. In coastal areas, climatic conditions may be suitable for *Ulva* cultivation, while the production of lignocellulosic plant biomass may be limited due to limited land and fresh water resources.

Here, we develop a methodology for modeling *Ulva* bioconversion to bioethanol. We apply the approach to the design of a two-step sequential *Ulva* fermentation process using metabolic models for ethanol production. In the first step, decomposed *Ulva* biomass is fed to *S. cerevisiae* for conversion into ethanol. In the second step, fermentation leftovers (including xylose) and the *S. cerevisiae* biomass resulting from first step are fermented by *E. coli* to produce additional ethanol.

We model three possible scenarios of *Ulva* bioconversion into ethanol. Model 1 (Table 1) consists of a one-step fermentation by wild type *S. cerevisiae*, with no ulvan decomposition or xylose uptake by *S. cerevisiae*. Model 1 predicts minimal ethanol yields of 15.6% of dry weight (dw), which is comparable to the

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Table 1. Scenarios for *Ulva* bioconversion simulations.

Model Number	Step 1 *S. cerevisiae*	Step 2 *E. coli*	Full ulvan decomposition and xylose uptake by *S. cerevisiae*
1	+	–	–
2	+	+	–
3	+	+	+

Table 2. *Ulva* chemical composition used in the modeling.

Total composition	Mean (%*w/w*)
Dry matter (%)	85.05
Ash	19.59
Protein	8.46
Lipid	7.87
Saturated fatty acids (SAFA) lipids (g/100g lipids)	68.97
Monounsaturated fatty acids (MUFA) lipids (g/100g lipids)	24.32
Polyunsaturated fatty acids (PUFA) lipids (g/100g lipids)	6.73
Soluble sugars	0.64
Uronic acid	9.97
Total dietary fiber	54.9
Insoluble fiber	34.37
Soluble fiber	20.53

experimentally-determined 14% yield (56). Model 2 consists of a two-step fermentation by *S. cerevisiae* and then by *E. coli,* with no ulvan decomposition or xylose uptake by *S. cerevisiae*. Model 3 consists of a two-step fermentation combined with full ulvan polysaccharide degradation and xylose utilization by *S. cerevisiae.* Both models 2 and 3 assume that *E. coli,* which we always assume to be the second fermentation stage, digests *Ulva* remnants, such as rhamnose, together with all *S. cerevisiae* biomass. Although the technology required for Model 3 has yet to be realized, it presents a theoretical *Ulva* bioconversion process for comparative purposes. Analysis of these three models using the methods explained below, provides estimates of carbon uptake, CO_2 emission, carbon utilization yields, and energy waste (w_2) during the bioconversion process.

Metabolic modeling methods

Metabolic analysis simulations of *Ulva* utilization and ethanol production were performed according to a commonly used Flux Balance Analysis (FBA) methodology. This method analyzes internal reaction fluxes based solely on simple physical-chemical constraints without requiring exact enzyme kinetic data. Specifically, such a methodology enables the prediction of biomass production rates based only on reaction stoichiometry and directionality. FBA-based approaches have a wide range of applications including phenotype analysis, bioengineering, and metabolic model reconstructions.[58–62] FBA is a sub-class of constraint-based modeling (CBM) frameworks that analyzes metabolic network behavior by utilizing known network structure (reaction stoichiometry) and metabolic flux constraints. The CBM framework linearly constrains possible reaction fluxes, leading to a range of feasible flux values for each reaction in the model. The detailed description of the analysis framework used in this study appears in Appendix 1. Specifically, we estimated minimal and maximal theoretical ethanol production rates under the assumption that bacterial growing rate is at least 95% of its theoretical maximum. All the simulations were performed using the COBRA Toolbox.[63] We used the Yeast5 model[64] for the *S. cerevisiae* simulations and the iJO1366 model[65] for *E. coli* simulations. The chemical composition of *Ulva lactuca* used in the modeling is shown in Table 2, the chemical composition of fibers was used as described in the references.[66–68] For further simplification, we assumed that all macromolecules have been depolymerized before the bioconversion process.

In all simulations, we were interested in two major outputs: ethanol yield and carbon utilization. The simulations were performed under anaerobic conditions assuming a 1 *g* of *Ulva lactuca* uptake for 1 *g* dry weight of *E.coli* in 1 h. Carbon utilization yield (*CUY*) is calculated as

$$CUY = \frac{C_{ethanol}}{C_{ulva}} \tag{19}$$

where $C_{ethanol}$ is the number of carbons converted by organism into ethanol, C_{Ulva} is the number of carbons in *Ulva*. The fraction of energy wasted on the bioconversion process (w_2) is calculated using Eqn (20):

$$w_2 = 1 - \frac{m_{ethanol} \cdot H_{ethanol}}{m_{ulva} \cdot H_{ulva}} \tag{20}$$

where $m_{ethanol}$ is the mass of ethanol produced, m_{ulva} is the mass of *Ulva* consumed, and *H* is the maximum heat of combustion. *H* for *Ulva* is 19 $kJ\ g^{-1}$ (45) and for ethanol is 30 $kJ\ g^{-1}$. 69 Our modeling approach enables rapid estimation of biomass conversion yields and suggests directions for optimization of *Ulva* bioconversion processing.

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Metabolic models simulations results and implications

Figure 4 depicts the conversion of *Ulva lactuca* into bioethanol using the two-step sequential fermentation by *S. cerevisiae* and then by *E. coli* as described in the Model 3 (Table 1). In this model, we assume full ulvan decomposition into monosaccharides, we also assume xylose utilization by *S. cerevisiae*. Rhamnose (and other components not consumed by *S. cerevisiae*) and the *S. cerevisiae* biomass resulting from the first fermentation step are then fermented by *E. coli* in the second step.

Simulation results are presented in Table 3. The highest ethanol yields are achieved when performing two-step sequential fermentation by *S. cerevisiae* and then by *E. coli*, with full ulvan decomposition and xylose uptake by *S. cerevisiae* (Model 3). The resulting yield predictions of 31–34.8% are close to the maximum theoretical ethanol production.[57] Currently, there is neither commercially available technology for full ulvan decomposition and nor *S. cerevisiae* strain capable of efficiently utilizing xylose. Removing ulvan decomposition and xylose uptake by *S. cerevisiae* (Model 2), reduces average ethanol yields to 25%. Therefore, ulvan decomposition and/or xylose utilization are important constituents of the *Ulva* bioprocessing process, and developing efficient technologies that further enable these processes would significantly contribute to the optimization of *Ulva* biofuel conversion. These findings indicate potential future directions for *Ulva* bioconversion improvement.

Comparing Models 1 and 2 shows a 7% yield advantage when performing the sequential two-step fermentation by *S. cerevisiae* and then by *E. coli* over the one-step fermentation by *S. cerevisiae* alone. This yield advantage results from the *E. coli* capacity to consume xylose and rhamnose. Performing the two-step fermentation reduced energy waste by ~34% during the conversion process ($w_{2model1}$ vs $w_{2model3}$). The modeling results shown in Table 3 have important implications for biorefinery design, and they project the biomass required for biofuel production.

We have analyzed and proposed a novel biotechnology process for *Ulva* polysaccharide saccharification, with

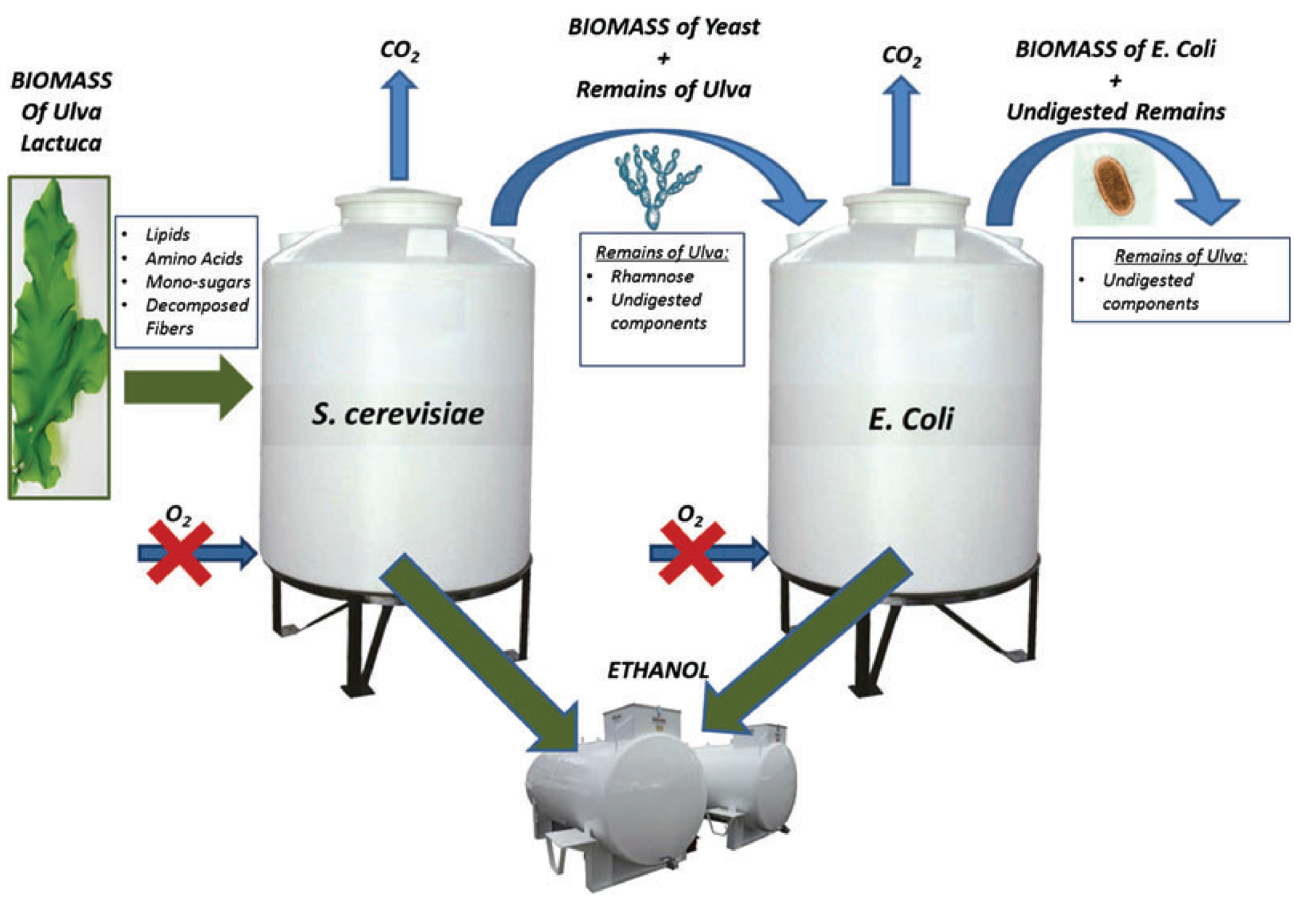

Figure 4. Schematic representation of the two-step bioconversion of *Ulva* feedstock into bioethanol - Model 3 (Table 1).

Table 3. Results of metabolic modeling of bioconversion.

	Carbon Uptake [%C]	Emission of CO2 [%C]	Ethanol Production (min-max) %dw	CUY (min-max)	w_2 (max-min)
Model 1	40.25%	11.28%	15.6–19.9%	19.00–24.23%	0.754–0.686
Model 2	68.17%	16.59%	22.8–27.2%	27.77–33.12%	0.640–0.571
Model 3	83.55%	22.24%	31–34.8%	37.75–42.38%	0.511–0.451

*Carbon utilization yield, as appears in Eqn (19).
** Energy wasted on the bioconversion process as appears in Eqn (19).

subsequent fermentation and production of ethanol. With the two-step fermentation technology as described in Model 2, we propose to significantly increase total ethanol yield from the currently reported maximum of 14%[41] to 22–27% using the appropriate available organisms at each step. This advancement would make macro algae *Ulva* competitive with lignocellulosic ethanol production, which has average yields of about 23–25%.[70] The FBA-based modeling approach introduced here provides numerical estimates of carbon utilization yield, total wasted energy and other interesting characteristics resulting from changing model parameters. This framework can be useful in studying other properties related to the process and is therefore of more general utility.

Sustainability and legal aspects of marine biorefinery design

Global policy debates on energy and climate have led governments in both post-industrialized and developing countries to adopt national legislative strategies aimed at reducing greenhouse gas (GHG) emissions, promoting clean energy production and enhancing energy security. Current and future national energy and environmental policies are likely to continue investments in the development of biofuel technologies as sustainable alternatives to fossil fuel exploitation. At the same time, first-generation biofuel production processes continue to raise social and environmental sustainability concerns, because of their reliance on agricultural lands, and their use of pesticides and potable water. National targets for bioenergy, along with subsidy programs to promote biofuel crops, have resulted in large-scale, albeit possibly unsustainable, biofuel production. The marine biorefinery model proposed here overcomes these issues to a large extent, but must also be put to a vigorous sustainability assessment, in order to fully understand its potential effects on the coastal ecosystem and on the livelihoods of indigenous and local communities. Sustainability of the proposed model would depend largely on its potential to generate socio-economic benefits while preserving biodiversity. In this regard, there still remain significant knowledge gaps on the full possible external effects of marine biorefineries, for example with respect to how the use of sea water for algae cultivation may impact the marine ecosystem, and the potential risks associated to the release of genetically manipulated algae into the natural environment. A comprehensive sustainability assessment of marine biorefineries is necessary, one that that moves beyond the traditional two-parameter focus on non-renewable energy use and GHG emissions, to incorporate economic, environmental, and social dimensions.

One of the most complex challenges regarding bioenergy is to understand what constitutes the sustainable production of biomass and biofuel, and how such processes can be measured and promoted. There has already been extensive work done, under various international, regional, and national multistakeholder initiatives, on the application of sustainability principles and criteria to biomass production. The development of sustainable biomass certification schemes is on the way. To date, most sustainability and certification frameworks for biofuels have focused on classical agricultural feedstocks such as soy, sugarcane, and palm oil. Since 2007, the Roundtable on Sustainable Biofuels (RSB), an international multistakeholder initiative based in Switzerland, has been working towards the adoption of a global set of sustainability standards applicable to any biofuel feedstock worldwide. The RSB's Principles & Criteria for Sustainable Biofuels (RSB-STD-01-001) contain guidelines on best practices in biofuel production and processing, specifying requirements for the certification of sustainable biofuel operations along the entire supply chain. Version 2 of the RSB Principles & Criteria came into effect on January 1, 2011 after several rounds of global stakeholder and public consultation. The four different operators subject to sustainability requirements under the RSB Principles & Criteria include producers and processors of feedstock, and producers and blenders of biofuels.

The RSB Principles & Criteria provide valuable insights into the complete set of social, environmental and

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economic issues that must be addressed in the planning and implementation of biorefinery concepts, providing stakeholders with a solid basis for informed and inclusive decision making. The 12 Principles contained in Version 2 of the RSB Principles and Criteria are outlined in Table 4.

Each RSB principle has an associated set of criteria and detailed compliance indicators.[72] The RSB also provides comprehensive guidelines on the implementation of the different impact assessments and stakeholder engagement processes required under various RSB Principles & Criteria. To gain better insight into the range of sustainability benefits of the coastal macro algae biorefinery, any potential implementation of the concept should be assessed under the RSB Principles & Criteria.

The question of protecting biodiversity (RSB Principle 7) merits particular attention, in light of new developments under the Convention on Biodiversity (CBD), an international agreement which aims to conserve biological diversity, to foster the sustainable use of the components of biological diversity and to achieve the fair and equitable sharing of the benefits arising from the use of genetic resources. The CBD came into effect in 1993 and currently has 193 member countries.

The many uncertainties related to the social and environmental impacts of intensified biofuel production prompted the 10th Conference of the Parties of the CBD to adopt *Decision X/37* (73) *on Biofuels and Biodiversity*. The decision sets out as its guiding principle the promotion of the positive and minimization or avoidance of the negative impacts of biofuels on biodiversity, and emphasizes the critical participatory role of indigenous and local communities in the elaboration and improvement of biofuel policy. A recent report[74] by the Secretariat of the CBD highlights the sustainable potential of small-scale biofuel production based on non-agricultural land, yet stresses the need to further develop sustainability standards, to conduct full life-cycle analyses and to engage all relevant stakeholders in decision-making on the development of biofuels.

In light of the growing importance of conservation and of the sustainable management of coastal environments, the location of marine biorefineries should only be undertaken with due consideration to the full range of environmental aspects, including whether the proposed infrastructure could aggravate coastal erosion, destroy native habitats, interfere with migratory patterns, or otherwise endanger fauna and flora. Equally important is an evaluation of the social aspects, which includes the potential displacement of local communities and businesses, and possible land-use conflicts. Local and state-level

Table 4. Version 2 of the RSB Principles.[71]

Principle		Regulation
1	Legality	Biofuel operations shall follow all applicable international, national and local laws and regulations.
2	Planning, Monitoring and Continuous Improvement	Sustainable biofuel operations shall be planned, implemented, and continuously improved through an open, transparent, and consultative impact assessment and management process and an economic viability analysis.
3	Greenhouse Gas Emissions	Biofuels shall contribute to climate change mitigation by significantly reducing lifecycle GHG emissions as compared to fossil fuels.
4	Human and Labour Rights	Biofuel operations shall not violate human rights or labor rights, and shall promote decent work and the well-being of workers.
5	Rural and Social Development	In regions of poverty, biofuel operations shall contribute to the social and economic development of local, rural and indigenous people and communities.
6	Local Food Security	Biofuel operations shall ensure the human right to adequate food and improve food security in food insecure regions.
7	Conservation	Biofuel operations shall avoid negative impacts on biodiversity, ecosystems, and conservation values.
8	Soil	Biofuel operations shall implement practices that seek to reverse soil degradation and/or maintain soil health.
9	Water	Biofuel operations shall maintain or enhance the quality and quantity of surface and ground water resources, and respect prior formal or customary water rights.
10	Air	Air pollution from biofuel operations shall be minimized along the supply chain.
11	Use of Technology, Inputs and Management of Waste	The use of technologies in biofuel operations shall seek to maximize production efficiency and social and environmental performance, and minimize the risk of damages to the environment and people.
12	Land Rights	Biofuel operations shall respect land rights and land use rights.

regulations on environmental and endangered species protection and land zoning rules must be fully considered. The RSB Principles & Criteria provide a cohesive and holistic framework for the treatment of these issues, amongst others.

Amongst prominent legal considerations is the sharing of benefits derived from biofuel production in light of the Nagoya Protocol,[75] a supplementary agreement to the CBD, which provides a legal framework for the fair and equitable sharing of benefits arising out of the utilization of genetic resources, within the scope of the CBD, Article 12 in the Nagoya Protocol.[75] While the Nagoya Protocol has yet to come into force, it is anticipated that the legally binding instrument will provide incentives to countries to conserve biological diversity and to enable the sustainable and equitable use of biological resources. The protocol primarily provides for fair and equitable benefit sharing between states, but also entails strong mechanisms to protect the rights of indigenous and local communities, in requiring that states support the development of community protocols. These are instruments developed by indigenous and local communities that outline their customary land and resources rights and also provide the terms and procedures for access to and fair and equitable sharing of benefits arising out of traditional knowledge associated with genetic resources. Under community protocols, indigenous and local communities may decide the terms under which their knowledge may be used, and their biological resources accessed. Thus, they can define the coefficients for *CSI*, Eqn (18), which we expect will assist in crop choice. Community protocols have already been successfully implemented by several communities in Latin America, Asia, and Africa, in a wide variety of contexts.[76,77] Providing an effective rights-based tool to communities to minimize risks of exploitation, to secure their continued access to land and resources, and to enhance their negotiating capacity *vis-à-vis* companies that wish to develop new products from biological resources, community protocols are likely to play a critical role in the sustainable development of marine biorefineries.

While this review does not aim to provide a full sustainability assessment of the macro algae biorefinery concept, some general observations can be drawn with respect to how macro algae relate to certain RSB principles in comparison to first and second-generation biofuel feedstocks. Most importantly in this regard, macro algae feedstock, in overcoming the food versus fuel concern associated to the majority of biofuel feeds, presents significant advantages linked to the protection and enhancement of local food security (RSB Principle 6) with associated benefits in the protection of soil, water and air resources (RSB Principles 8, 9, 10) and CO_2 mitigation (Principle 3). Moreover, as macro algae production does not compete for arable land or potable water, it is more likely to support rural and social development (RSB Principle 5) than the production of previous generation feedstocks, particularly through the implementation of small-scale cultivation systems such as the low-tech system proposed here, which may be assembled and maintained by local communities in developing countries. As for issues of legal conformity (RSB Principle 1), planning, monitoring and improvement processes (RSB Principle 2) and respect for labor, human and land rights (RSB Principles 4 and 12), sustainability performance will evidently depend on the specific geographical setting, political context, actors involved, processes followed and agreements concluded. Similarly, no general conclusions can be drawn with respect to the impact of macro algae cultivation on the conservation of biodiversity (RSB Principle 7), the maximization of social and environmental performance, or the minimization of risks to the environment and people (RSB Principle 11), all of which will depend on the specific context of implementation.

Marine biorefinery design for a rural town in India

The goal of this section is to demonstrate the implementation of the developed models for the design of the *Ulva* feedstock based biorefinery to supply transportation fuel needed for an average town in rural India.

In 2005, the annual per capita transportation fuel consumption in India was 9.9 L.[78] Analysis of the population distribution[79] shows that the average town in coastal rural India has 15 000–25 000 inhabitants. In this model, we assume that economic growth drives the growth in personal fuel consumption to 20 L of gasoline per person per year. Therefore, the local biorefinery should supply about 400 000 L of gasoline equivalent per year. If bioethanol is chosen as a biofuel, then ~627 000 L per year production capacity is needed to produce bioethanol with the equivalent combustion heat value as 400 000 L of gasoline.

Results from modeling of two-step fermentation by the *S. cerevisiae* and *E. coli* of *Ulva* imply that the biorefinery will consume 2000 ton of *Ulva lactuca* per year. Recent work on *Ulva* intensive cultivation reported production rates of up to 45 ton DW per ha.[56] Therefore, a total area of 45 ha of ponds will be needed to supply 100% of transportation needs for this model town. Our results are of particular interest, as biorefineries of 627 000 L (167 000 gallons) per year capacity are already available today on the market.

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Nevertheless, multiple barriers still exist for efficiently building biofuel capacity in developing countries. Successful technology adaptation requires a deep understanding of the local social, political and infrastructural/technological environment.[80] Food security, employment policies, land conflicts, environmental policies, and local business networks can all affect capacity building.

Future directions in biorefineries development

There is an urgent need for the biofuel sector to get an estimate on energy expenses for feedstock collection, transportation and final product distribution. Currently, there is a rule of thumb that ~25 miles is the optimal size of area for feedstock collection. Thermodynamic and economic studies are needed to find the optimal collection distances as a function of local fuel demand and conversion technologies. Very few studies comparing the efficiency of biorefineries at different scales are available. These studies are essential for better future design. In this work, we showed the possible direction for optimization of biomass bioconversion to biofuels using emerging metabolic engineering approaches.

Due to the yields and sustainability advantages measured in use of land, potable water and chemicals, macro algae are an excellent feedstock for transportation biofuels. The cultivation and decomposition technologies, however, require further improvement. Recent reports on engineered microbes, which digest the majority of brown macro algae polysaccharides, have shown that macro algae are a practical and available feedstock for biofuels. Green macro algae (*Ulva sp.*) have an enormous advantage in higher growth rates. We believe that modern synthetic biology and molecular engineering approaches can lead to low cost biological methods for the decomposition of *Ulva* to fermentable sugars.

Combined agricultural, downstream processing and policy models are urgently needed to provide planning tools to multiple communities interested in sustainable energy sources. Today, however, these models remain separated in their professional subfields. Combining agricultural, process engineering, and policy models is possible, and we believe that the best way to reduce to practice rapidly is through-small scale projects with the highest immediate impact. Rural areas in developing countries are an excellent opportunity in this field. In Fig. 5, we show the conceptual flow for biorefineries design.

Finally, a great challenge for any new technology is field implementation. Bringing new energy technologies to rural communities in developing countries has economic and social challenges. Specific policy development and the integration of distributed biorefineries into coastal community business networks will be required. The implementation of initial pilot facilities will significantly reduce barriers to entry as well as and train personnel.

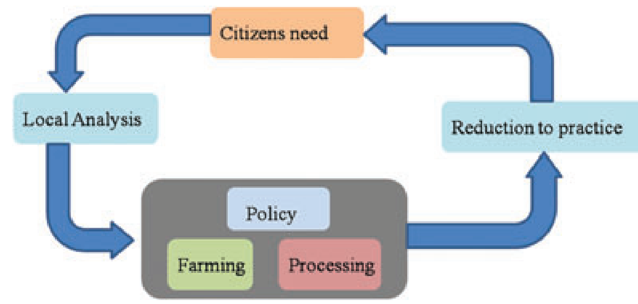

Figure 5. Biorefinery design, modeling, and implementation.

Conclusions

Biofuel programs are under development globally. The successful implementation of biofuel projects will require detailed modeling and simulation. In this review we present the integrated approach of biorefineries design, combining thermodynamic modeling of biorefineries size and distribution, metabolic modeling of biomass into biofuel conversion, and legislation policy implications for the design of local biorefineries with an emphasis on developing economies. We exemplify this integrated approach by a discussion of marine biorefineries based on macro algae *Ulva* and present here: modeling and analysis of several scenarios for *Ulva* conversion into bioethanol by one or two-step microbial fermentation; demonstration a high-level model of biorefinery design for an average town in rural India; and adaption of the sustainability criteria for marine biorefineries design. We believe that our combined design approach will promote the implementation of sustainable distributed biorefineries in developing countries.

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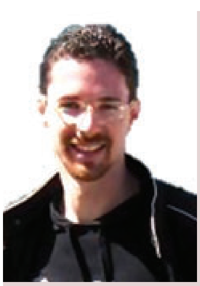

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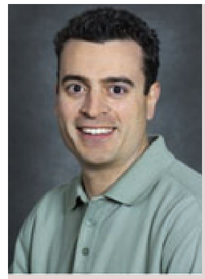

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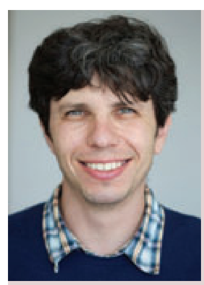

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Gregory Linshiz works at the Joint BioEnergy Institute (JBEI) - Lawrence Berkeley National Laboratory. In his research, he integrates diverse approaches and combines ideas from Biology, Computer Science and Engineering to find creative solutions for biofuel production and development of new automated platforms for Synthetic Biology.

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