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Seaweed biorefineries: exergy efficiency, fermentation and sustainability implications; example of potential production of bioethanol from *kappaphycus alvarezzi* in Philippines

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Abstract:

Biomass to fuel programs are under research and development worldwide. The largest biomass programs are underway in the USA and in Europe. In the coming decades, however, developing countries will be responsible for the majority of increases in transportation fuel demand. Although the lack of existing large-scale infrastructure and primary resources preclude oil refining in the majority of developing countries, this provides an opportunity for the rapid implementation of small scale distributed biorefineries to serve communities locally. Currently, most of bioenergy production is based on the terrestrial biomass, which, however, has a major limitation of land availability. In parallel vein, recent results indicate the potential of the marine biomass, seaweeds, potential as a next generation bioenergy crop that does not require arable land, drinking water and fertilizers used in the terrestrial agriculture systems. The use of seaweeds as a feedstock for bioenergy production is very limited today mostly because seaweed farming takes place in the remote rural area in the medium and low-income countries, where the processing technology is not available. Moreover, the efficiency of seaweed conversion into transportation biofuels is not optimized and thus the estimations of the productivity are not as well understood as in the case of the first and second generation bioenergy crops. In the first part of this paper, we report on the high-level exergy model of the biorefinery. This model allows to find the parts of the biorefinery that can be optimized in a way the whole system gets the largest benefit. In the second part, we report on the metabolic model of seaweed fermentation into bioethanol, a platform chemical that can be used for a chemical and biofuel industries. The models allow for a rapid prediction of various fermentation scenarios to identify the most efficient conversion process, given the chemical composition of the biomass. In the third part, we analyze the potential of bioethanol production from off-shore cultivated macroalgae from the *Kappaphycus* family. We show that using a two-step fermentation first by yeast, *Saccharomyces cerevisiae*, and second by a bacterium, *Escherichia coli*, it is possible to generate additional revenue streams to the rural farms that are involved in the seaweed cultivation in Philippines. We also show that using currently available cultivation and fermentation methods it is possible to supply 100% of Philippines current demand on the transportation bioethanol from the seaweed industry, if the cultivation area is increased only 3 times from its current size.

Keywords: Energy system design, exergy, fermentation modelling, sustainable development, biofuels, bioenergy, Philippines bioethanol.

1. Introduction

Economically efficient, socially and environmentally sustainable conversion of biomass into valuable products is a major contemporary challenge for science, governments and businesses worldwide [1]. Transportation fuels, electricity, heating, cooling, drinking water, food, animal feed, chemicals, and materials are all potential products of biomass conversion. The system that integrates the conversion of solar energy and carbon dioxide via photosynthesis into biomass, biomass harvesting, processing, and distribution of derived chemicals and bioenergy is coined biorefinery [2]. Design of a sustainable biorefinery, which will generate sustainable food, fuels and chemicals is a complex task and is largely influenced by local raw material supplies, advances in multiple technologies and socio-economic conditions [3]. The key questions in the biorefinery design are where the systems should be installed and how to choose the feedstocks and processing and conversion technologies [4].

1.1. Biorefineries demand in medium and low-income countries

The major growth in the demand for food and liquid fuels in the coming decades will be predominantly due to the rising standard of living and consumption in the medium and low income countries [5]. Although In the Organization for Economic Co-operation and Development (OECD) countries, Gross Domestic Product (GDP) and population growth are not linearly correlated to primary energy consumption, in the medium and low income countries GDP growth requires an increase in consumed energy [6]. The World Energy Council predicts that India and China will overtake developed countries in transportation fuel consumption by 2025 [7]. Moreover, the increasing standard of living put additional pressure on the food and energy production systems in these areas. Therefore, the largest need for the new refinery infrastructure is in medium and low income countries [8]. This new infrastructure, if carefully designed, could lead to the sustainable development in the major parts of the word. A major parts of the refinery design is the choice of raw materials, processing technologies and efficient logistics [4].

1.2. Biofuels feedstocks options/problems

Current strategies for food production and renewable energy generation reply mostly on the classic terrestrial agriculture. However, *a key issue for biomass for energy production is land availability* [2]. Furthermore, concerns over net energy balance, potable water use, environmental hazards, and processing technologies question the cereals crops and lignocellulose biomass to provide sustainable answer to the coming food and energy challenges [9]. The cost-effective cultivation and dehydration difficulties currently prevent broad scale microalgae technologies implementation [10]. The on-shore cultivation of microalgae for energy, though promising, is currently impossible due the costs of required infrastructure and negative net energy balance on the entire systems [11]. However, an expanding body of evidence has demonstrated that marine macroalgae can provide a sustainable alternative source of biomass for sustainable food, fuel and chemicals generation [12-16]. Macroalgae, which contain very little lignin and do not compete with food crops for arable land or potable water, have only recently fell under the research radar as additional candidates for future sustainable food and transportation fuel feedstocks [12-16].

1.3. Opportunities for seaweed biorefineries

Macroalgae biomass use for food, chemicals and fuels production is currently based on cultivation, decomposition, separation and fermentation. Various green, red and brown macroalgae species are under evaluation for inland and off shore cultivation [15-17]. Intensive macroalgae farming systems have been previously reported [14]. However, achieving intensified biomass yields in on-shore macroalgae cultivation ponds requires additional electrical power for mechanical mixing during active photosynthesis [14]. Since this additional power is only required during the day, we previously proposed design for solar photo-voltaic (PV) systems to generate the required energy, thus integrating solar electrical power generation with intensive macroalgae cultivation [18]. We suggested a network of modular marine biorefineries, integrated into distributed energy networks, where electricity is supplied from a large central PV facility, but biofuels and food ingredients are produced locally [18].

Energy efficient processing of biomass to food and fuels intermediates is critical for the successful future application of macroalgae biomass. Production of acetone, ethanol and butanol from seaweeds has been demonstrated [15,19]. Moreover, significant advances for macroalgae derived sugars for biofuel were recently achieved using synthetic biology tools [20]. However, in all mentioned processes, macroalgae biomass was first dried and then decomposed to basic biochemical using thermal and chemical methods and then fermented using a single culture systems [21,22]. These existing processes, however, are energy intensive and single culture fermentation systems are fragile, and thus and will sustain manufacturing of low cost commodities such as food and biofuels; therefore, alternative strategies for processing are needed.

Multiple opportunities for macroalgae biorefineries implementation exist in the medium and low income countries, where most of the global seaweeds are produced today. We demonstrated this approach by a design of an on-shore cultivated macroalgae biomass based biorefinery to supply biofuels and feed to an average town in coastal India [23]. The on-shore cultivation systems, however, require land and energy supply, which is not always available in the remote rural locations where seaweeds are usually farmed. The off-shore cultivation of seaweeds is currently employed in Indonesia, Philippines, China, India and Tanzania, mostly for the carrageenan and agar production. Interesting, accordingly to the recent Food and Agricultural Organization of the United Nations report of the seaweed for carrageenan, only 8-30% of the seaweeds are used for the processing and the rest of the biomass is treated as waste [24]. As the major seaweed cultivation today is done by on the family level poorest farmers, the additional revenue streams and thus increase efficiency of the already excising cultivated seaweed biomass could provide additional income to the families [24]. The seaweeds, however, and the outputs of the processing are not converted today in chemical such as bioethanol that can be used for both food industry and for transportation biofuels.

The goal of this paper is to develop fundamental governing equations that describe the seaweed biorefinery efficiency and to evaluate a potential to bioethanol production from the red commercial species *Kappaphycus alvarezzi*. *K. alvarezzi* is of particular interest for this paper as this species is currently the most widely cultivated macroalgae species [24]. In Section 2 of the paper describes the high-level model on the exergy flow analysis of a biorefinery in a general case. In Section 3 we described the metabolic model of seaweed conversion to bioethanol. In Section 4 we demonstrate the concept of seaweed biorefinery for bioethanol production in the seaweed farms in Philippines, one of the world largest seaweed producers.

2. Exergy model of biorefineries

Consider the biorefinery system in the production scale. The system converts solar and mechanical energies into the concentrated energy products, such as food, energy and platform chemicals. If the system performance is measured in the units of exergy, in the most general case (**Figure 1**), the inputs to the process are represented by an exergy stream of solar energy supply (e_{s_e}), mechanical energy supply, (e_{m_e}), materials (e_m) capital inflow (e_k) and human labor (e_l), and information, represented by eco-exergy (e_{eco}). The outputs are the delivered exergy contained in food (e_f), useful energy, such as biofuels (e_e), and platform chemicals (e_c), exergy rejection to the environment (e_{en}), materials waste (e_w), and eco-exergy, information, loss (or gain) ($e_{eco\text{-}c}$). As both physical and information exergies are conserved in these systems, the system will experience a continuous physical and information exergies losses. The efficiency of such biorefinery can be calculated as described in **Equation 1**:

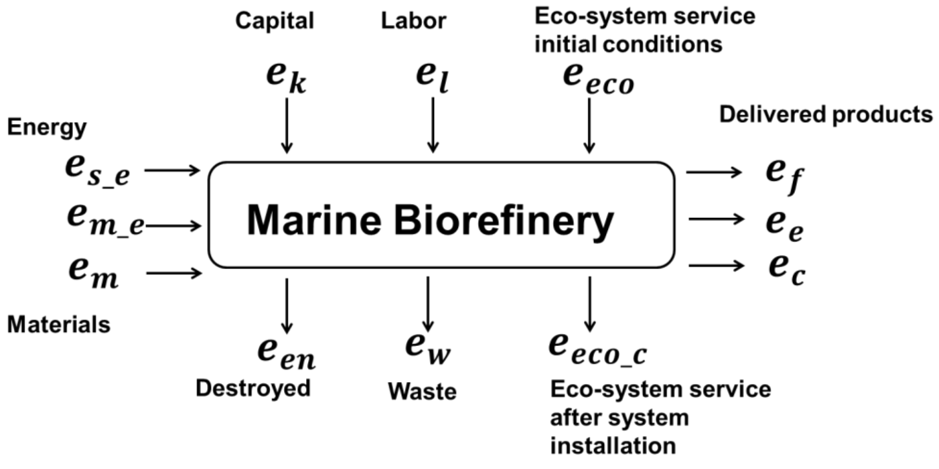

Figure 1. High level exergy model of a biorefinery

$$\eta = \frac{e_f + e_e + e_c}{e_{s\_e} + e_{m\_e} + e_m + e_k + e_l + e_{eco}} \tag{1}$$

The exergy diagram for the seaweed biorefinery appears in **Figure 2**. The major, currents of exergy include the solar exergy, converted by plants to biomolecules; fossil fuels for fertilizes manufacturing (if required); deployed eco-system services and capital. The three major sources of exergy losses are 1) loss on photosynthesis (solar energy conversion to biomass), 2) losses on processing: cultivation, transportation, conversion, distribution, final product combustion biodiversity and 3) eco-exergy losses. The goal of the designer is to reduce the exergy losses. In the next chapter, we will describe and approach to reduce the exergy losses during the bioconversion process, when a biomass, for example, seaweed is converted into bioethanol.

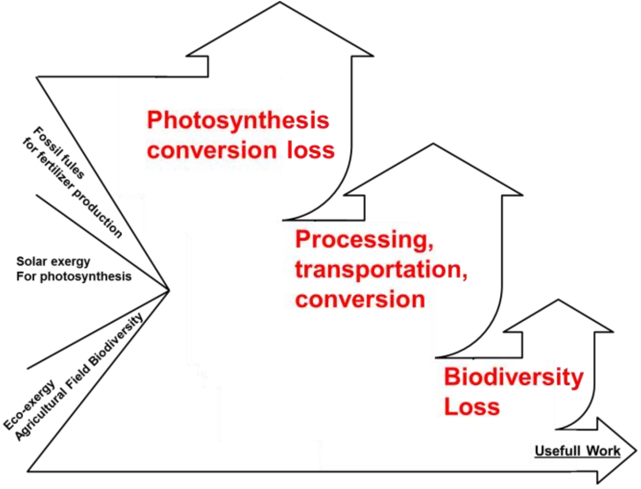

Figure 2. Exergy diagram for a biorefinery

3. Metabolic modelling of seaweed conversion into bioethanol

One of the major decisions in biorefinery design is the construction of the biomass processing unit, responsible for conversion of harvested biomass into desired products. The efficiency of the unit will predicate the exergy rejection to the environment (e_{en}), materials waste (e_w). If the conversion is biological, this unit is composed of one or several possibly genetically modified organisms, who extract the molecules of interest as by-product of their growing activities. The choice of these organisms is a complex task and is largely influenced by the expected feedstock, since not all the organisms can consume the entire feedstock biomass, and by the desired product, since not all the organisms can produce the target molecules.

3.1. Biomass processing unit construction

Seaweed species, which is our biomass feedstock, are usually comprised from high amounts of various fibres, like cellulose, hemicellulose reaching up to 30% in *K. alvarezzi* [25]. Other prevailing molecules are carbohydrates, amino acids and fatty acids, reaching respectively around 27%, 16% and 1% in *K. alvarezzi*. Indeed, such feedstock is very heterogeneous and finding right setup for the biomass processing unit is not trivial. To simplify this task we can use various mathematical simulations approaches prior to creating the process *in-situ*, such as FVA technique described in the next section.

The natural candidate for production of bioethanol, which is our molecule of interest, is *S. cerevisiae*. However, it poorly utilizes significant part of the seaweed carbohydrates such as *xylose*, *rhamnose* and *galactose*, leading to low carbon utilization yield. One option to overcome this matter and to improve the bioethanol yields is to genetically modify *S. cerevisiae* to improve sugar uptake mechanisms. Although studies to fulfil this option conducted in many laboratories over the past years, it remains an open challenge [26]. Here we describe an alternative two-step fermentation approach to deal with this issue (**Figure 3**), originally reported in [23]. In the first step, decomposed seaweed biomass is fed to *S. cerevisiae* for conversion into ethanol. In the second step, fermentation leftovers and the *S. cerevisiae* biomass resulting from first step are fermented by *E. coli* to produce additional ethanol. In the next paragraph we report on a computational method to estimate the conversion of a common commercial red seaweed *K. alvarezzi* into bioethanol using two step fermentation process.

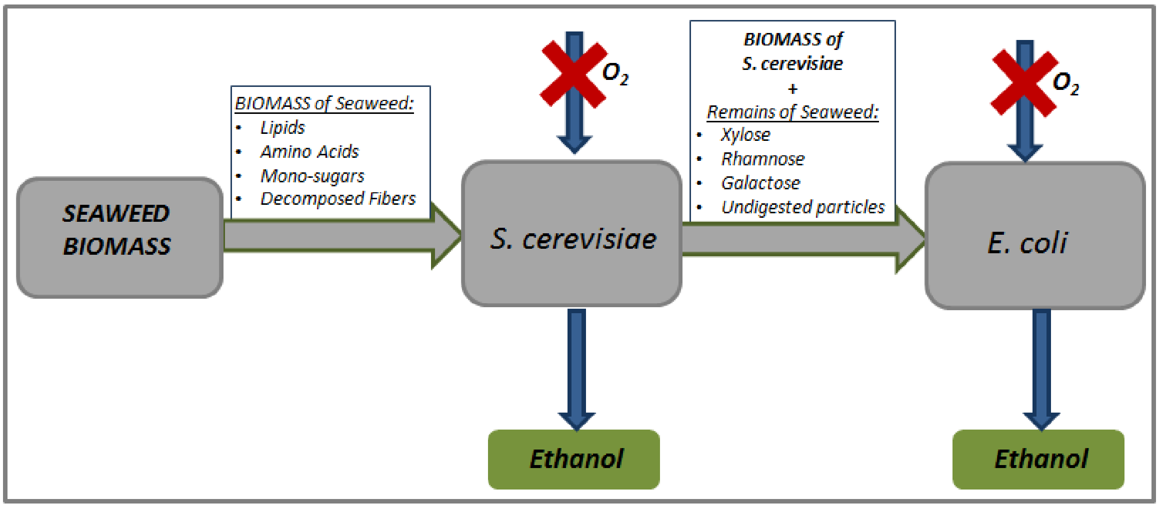

Figure 3: Schematic representation of the two-step bioconversion of seaweed feedstock into bioethanol.

3.2. Flux Balance Analysis of Seaweed to Bioethanol Conversion

Commonly used computational approach to predict metabolic organism behaviour is Flux Balance Analysis (FBA) technique. This method analyses internal reaction fluxes based solely on simple physical-chemical constraints without requiring exact enzyme kinetic data. Specifically, this 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 [27-31].

There are two constraint types widely used in various FBA-based methods: (i) mass-balance constraints imposed by network stoichiometry (**Equation 2**), and (ii) maximal/minimal feasible reaction flux constraints (**Equation 3**). Where S is a stoichiometric matrix, in which $S_{m,r}$ corresponds to stoichiometric coefficient of metabolite m in the reaction r, and $\vec{v}$ is a vector of reaction.

$$S \cdot \vec{v} = 0 \tag{2}$$

$$v_r^{LB} \leq v_r \leq v_r^{UB}, \forall r \in reactions \tag{3}$$

Although the v^{LB} and v^{UB} are unknown and therefore defined to be set to [-*Inf*;*Inf*] for most of bidirectional and to [0;*Inf*] for unidirectional reactions, we can still bind the solution space by limiting the growth media uptake rate. In our specific case the knowledge of actual media uptake rate is not critical, because we are not interested in reaction rates, but rather in total conversion yield (in %) of dry algal biomass into ethanol. Therefore, we assumed the uptake rate of 1gDW*h of *K. alvarezzi* and calculated the *Kappaphycus*-to-*Ethanol* conversion yield accordingly.

The FBA framework is assuming that the modelled organism metabolic network is regulated so as to maximize some cellular function under the predefined set of constrains (**Equation 2, 3**). The most common cellular target for unicellular organisms is maximization of organism growth rate, thus leading to optimization framework presented by **Equation 4**. Where $v_{biomass}$ is an artificial growth reaction converting all the organism biomass constituents into units of biomass.

$$\max_{\vec{v}} v_{biomass} \tag{4}$$
$$s.t.:$$
$$\begin{bmatrix} S \cdot \vec{v} = 0 \\ v_r^{LB} \le v_r \le v_r^{UB} \end{bmatrix}$$

Indeed, there may be multiple solutions for vectors of reaction fluxes both satisfying all the predefined constrains and maximizing organism growth rate. This means that each non-biomass reaction and particularly, ethanol-producing one may have a range of possible values. This range is estimated using Flux Variability Analysis (FVA) formulation [32], as presented in **Equation 5**:

$$\max/\min_{\vec{v}} v_{ethanol} \tag{5}$$
$$s.t.:$$
$$\begin{bmatrix} v_{biomass} \; is\ a\ solution\ to\ Eq.\ 4 \\ S \cdot \vec{v} = 0 \\ v_r^{LB} \le v_r \le v_r^{UB} \end{bmatrix}$$

During the two-step simulations we performed FVA analysis twice. First, we take seaweed biomass as media and inspect the upper and lower fluxes for ethanol-producing reaction in the metabolic model of first organism. Second, we take the undigested remains of original media and the biomass of the first organism as media for the second organism and inspect the ethanol-producing reaction in it (**Figure 3**).

All the simulations were performed using the COBRA Toolbox [33] . We used the Yeast5 model [34] for the *S. cerevisiae* simulations and the iJO1366 model [35] for *E. coli* simulations.

3.3. Computational analysis of *K. alvarezzi* fermentation

We evaluated ethanol production for the following four organism setups: two possible orderings of *S. cerevisiae* and *E. coli* and two single organism fermentations. In all simulations, we were interested in two major outputs: ethanol production yield and carbon utilization yield. The simulations were performed under anaerobic conditions assuming a 1g of seaweed uptake for 1g dry weight of organism in 1hr. Carbon utilization yield was calculated as ratio of carbons in ethanol to carbons in media. To simplify the computational simulations we assumed that all macromolecules have been depolymerized before the bioconversion process.

The summary of chemical composition of *K. alvarezzi* used in our modelling is shown in Table 1 while details can be found in [25,36,37].

Table 1. K. alvarezzi chemical composition used in the modelling

Total composition	Mean (%w/w)
Moisture	6.43
Fibre	29.4
Ash	19.7
Protein	16.24
Lipid	0.74
Carbohydrate	27.4
Ascorbic acid	0.107
Vitamin A	0.00087
Minerals	3.65

As demonstrated in Table 2, we predict to achieve maximal bioethanol production rate in two-step fermentation setup with *S. cerevisiae* as first organism in the process (148-156 gEthanol/Kg DW *K.alvarezzi*) . In such setup we expect ~46% product increase (decrease in the exergy content of the wasted products) comparing to *S. cerevisiae* alone. Notice that switching the order of organisms is not beneficial, since *E. coli* consumes all available *K. alvarezzi* components producing less ethanol (105-112 gEthanol/Kg DW *K.alvarezzi*) than the two-step process and leading to predicted zero-growth rate of the *S. cerevisiae* on the leftovers.

Table 2. Simulation results of fermentation of K. alvarezzi biomass

Configuration	***E. coli*** **growth [h^{-1}]**	***S. cerevisiae*** **growth [h^{-1}]**	**Min Ethanol**		**Max Ethanol**	
			Production [g/Kg]	**Carbon Utilization [%]**	**Production [g/Kg]**	**Carbon Utilization [%]**
S. cerevisiae	---	0.0138	85.4483	10.2%	100.3559	12.0%
E. coli	0.0441	---	105.7642	12.6%	112.0598	13.4%
S. cerevisiae ⇒ *E. coli*	0.0308	0.0138	148.7685	17.7%	156.1645	18.6%
E. coli ⇒ *S. cerevisiae*	0.0441	0.0001	105.7623	12.6%	115.4626	13.8%

4. *Kappaphycus* based biorefinery design for rural farms in Philippines

In the previous two paragraphs we showed the high-level modeling of the biorefinery and also introduced a method for bioconversion optimization of seaweeds into bioethanol using FBA. In this part of the paper we show the potential of the seaweeds biorefinery to generate additional value to the seaweed growers and other coastal communities in Philippines. We chose to exemplify the potential implementation of seaweed based biorefineries on the example of Philippines, the rapidly developing country that is one of the world largest producers of seaweeds [24]. In this study, we analyze the bioconversion of the common to Philippines seaweed *Kappaphycus* into bioethanol. Although, if used for the transportation biofuel, the energy density of bioethanol is lower than gasoline or diesel, the final steps of ethanol purification is well established in the small and large scale.

In 2006 Philippines passed the Biofuel Act, making it mandatory to use bioethanol in fuel blends. Initially, four potential crops were identified as feedstocks for the local bioethanol industry: sugarcane, corn, cassava and sweet sorghum. However, almost ten years after the Biofuel Act was passed, Philippines still produce only 30% (85 million liters as for 2012) of their local demand, importing rest of the bioethanol [38]. Moreover, as we mentioned before terrestrial biomass production is limited due to the limited land availability, requirement for fresh water, fertilizers and potential competition with the food production.

In previous works, using life-cycle analysis, we have shown the advantage of macroalgae feedstock for biofuel production potential in comparison with corn and cassava fresh roots in terms of land, potable water, fertilizer and herbicide usage [23]. Philippines have almost 50 years history in commercial seaweed farming, with *K. alvarezzi* as a major cultivated crop [24]. The industry, which mostly target seaweeds for carrageenan processing , have already generated thousands of jobs and improved the quality life to multiple families in the rural coastal areas. The current area for seaweed farming Philippines in the major producing regions of ARMM is about 24,000 ha with the potential expansion to 103,000 ha [24]. The average productivity using current cultivation methods in Philippines is 31 ton DW ha^{-1} $year^{-1}$ [24]. Several previous studies have investigated the conversion of *K. alvarezzi* into ethanol by a single step process [39, 40].

To estimate the bioethanol production from *K. alvarezzi* in Philippines by a two-step process, we used the metabolic model from Section 3 (Table 3) and Equation 6:

$$BPP = yield \cdot production \tag{6}$$

Where $BPP\ (lit\ ha^{-1} year^{-1})$is the predicted bioethanol production, $yield\ (kg\ DW\ ha^{-1} year^{-1})$ is an average seaweed yield and $production\ (lit\ ethanol\ kg\ DW^{-1}\ )$ is the conversion efficiency of dry seaweed into bioethanol through fermentation.

Table 3. Bioethanol production potential (BPP) of K. alvarezzi biomass ha^{-1} $year^{-1}$ given the current biomass productivity of 31 ton DW ha^{-1} $year^{-1}$. Results are based on FVA simulations

Fermentation configuration	***Min Ethanol*** [lit ha^{-1} $year^{-1}$]	***Max Ethanol*** [lit ha^{-1} $year^{-1}$]
S. cerevisiae	2649	3111
E. coli	3279	3474
S. cerevisiae ⇒ *E. coli*	4611	4841
E. coli ⇒ *S. cerevisiae*	3279	3579

If total transportation bioethanol demand in Philippines is 283 million liters per year [38], ~60,000 ha will be required on the national level to cultivate *K. alvarezzi* using current methods for the reported two-step fermentation process, which predicts up to ~ 4841 lit of ethanol production ha^{-1} $year^{-1}$. This yields are close to the maximum yields of ethanol predicted in the previous theoretical studies that used only the sugar to ethanol ratio calculation [39].

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For a single farm or local cooperative, bioethanol production from currently wasted seaweed biomass material could generate additional profit streams. For example, two representative farms in Zamboanga, Philippines, reported on 2.85 ton DW $year^{-1}$ (cultivation area of 0.05ha, Farm A) and 8.5 ton DW $year^{-1}$ (cultivation area of 0.27ha, Farm B) [24]. If 70-92% of the produced biomass which is lost today is converted into bioethanol, this can generate additional 295-409kg of ethanol for Farm A and 880-1220 kg of ethanol for Farm B.

Importantly, until now seaweed farming, has contributed to improving the socio-economic status of coastal communities in the Philippines. The farms generate employment for tens of thousands of coastal families; provide diversified livelihoods to meet basic family needs such as food, shelter, education of children, and health care, among others; enhance community cohesion through cooperation among farmers. In addition, seaweed farming was shown to strengthen stewardship of marine environment and resources; promoting development of and enhancing viability of small and medium enterprises [24]. We believe the development of low cost processing systems to convert seaweeds and the waste of their processing into platform chemical will further contribute to the sustainable development in the poor rural areas. Moreover, the development of additional products from seaweeds, such as bioethanol through fermentation, could address the current challenges of the industry such as low income of farmers, which is mostly because of seasonal and unstable production, and poor market linkages that deprive seaweed farmers of benefits of the seaweed value chain.

Conclusions

In this paper we proposed the high-level model of the new type of biorefineries for low and medium-income countries, based on seaweeds feedstock. Seaweed biorefineries could provide the sustainable alternative to the fossil fuels and terrestrial biomass feedstock in multiple coastal areas. Development of seaweed based biorefineries could preserve the arable land and drinking waste by moving the biomass production off-shore. We have shown that using currently available computational methods it is possible to predict the potential of additional revenue streams generation to the seaweed farmers by the production of bioethanol. Addition of this revenue stream could significantly reduce the current industry waste and also open new opportunities for the further sustainable industry growth.

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