Agr. Nat. Resour. 55 (2021) 893–903

AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Simulation for integrating bioethanol production in existing commercial agar extraction plant toward zero waste technology

Teuku Beuna Bardant[a,†,*], Eka Triwahyuni[a], Muryanto[a], Roni Maryana[a], Yan Irawan[a], Ifah Munifah[b], Pujoyuwono Martosuyono[b], Rodiyah Nurbayasari[b], Sugiyono[b], Uju[c], Ekowati Chasanah[b,†], Yanni Sudiyani[a]

[a] *Research Center for Chemistry, Indonesian Institute of Sciences, Banten 15314, Indonesia*

[b] *Research Center of Marine and Fisheries Product Processing and Biotechnology Agency for Marine and Fisheries Research, Ministry of Marine Affairs and Fisheries, Central Jakarta 96002, Indonesia*

[c] *Department of Aquatic Products Technology, Bogor Agricultural University, Bogor 16680, Indonesia*

Article Info

Article history:
Received 8 July 2021
Revised 15 September 2021
Accepted 19 October 2021
Available online 31 October 2021

Keywords:
Energy efficiency,
Enzymatic hydrolysis,
Gracillaria,
Sustainable process,
Waste valorization

Abstract

Filter cake (FC) from an agar extraction plant has potential as bioethanol production feedstock due to its abundance and not requiring pretreatment. Simulations were conducted using three modified saccharification/hydrolysis processes utilizing FC as feedstock to define the required material and energy. FC samples from two agar extraction plants were characterized. Then, the promising FC was used as feed in a laboratory-scale test in three modified saccharification scenarios. Conversion values from the laboratory test were used in simulations involving a 500 L fermentation tank to calculate the required material and energy for commercial-scale application. FC with 16.4% dry weight of cellulose was used in the simulation. The highest ethanol concentration (2.5% by volume, v) was produced using hydrolysis with prior drying, but required 1291.9 kWh/L ethanol. The main electricity consumption for the other two scenarios was only for the agitation motor in the hydrolysis process. Unseparated filter aid material (FAM) scenario produced 2.2% v ethanol but only required 244.9 kWh/L ethanol, which was the least compared to the other treatments. The unseparated FAM scenario also required the least heat for distillation (134.51 MJ/L); however, this was only 4 MJ less than for the extractive hydrolysis scenario that required the most heat. The unseparated FAM scenario was the most promising scenario for bioethanol production. Purification of carrageenan and alginate could be used to obtain ethanol. Separating the FAM before fermentation made it possible to use the glucose solution for other potential financially viable applications, such as sweeteners. This study provided prospective insight for seaweed and agar industrialists regarding the intensification or expansion of this approach for other seaweed-based chemicals.

† Equal contribution.

* Corresponding author.
E-mail address: qaismajnun@gmail.com (T.B. Bardant)

https://doi.org/10.34044/j.anres.2021.55.5.20

Introduction

The utilization of biomass waste as bioethanol feedstock has been studied using cocoa pod shell (Shet et al., 2018), spent coffee grounds (Wongsiridetchai et al., 2018), coconut husk (Vaithanomsat et al., 2011), cassava stem (Niyomvong and Boondaeng, 2019), cassava peels (Kongkiattikajorn and Sornvoraweat, 2011) and sugarcane bagasse pith (Sritrakul et al., 2017). Even though these forms of biomass waste have potential due to their abundance, the necessary pretreatment processes are still the main challenge to make them economically feasible and many studies have addressed this by minimizing the pretreatment. Initial approaches used tough enzymes and microbes in saccharification and fermentation to lower the required specification of pretreatment product via optimizing agitation in saccharification (Rodmui et al., 2008), using co-culture yeast (Kongkiattikajorn and Sornvoraweat, 2011), using thermos-tolerant yeast and repeated batch fermentation in a nonsterile system, (Pimpakan et al., 2012) or using a halotolerant hybrid by intergeneric protoplast fusion of *Saccharomyces cerevisiae* (Limtong et al., 1998). More direct approaches were applied by developing less-demanding pretreatment using dilute acid (Sritrakul et al., 2017; Shet et al., 2018) or keep the original alkaline pretreatment but including the option to sell other products such as oligosaccharides (Wongsiridetchai et al., 2018).

Seaweed waste as feedstock has similar advantages to the above biomass waste types of not competing with food sources and being freely available and abundant (Goh et al., 2010). In the extraction process, dried seaweed can be treated with hot alkali solution to reduce the sulphate content and to improve the gel strength to produce agar or carrageenan (Li et al., 2008; Meinita et al., 2017). This has the added advantage since the required pre-treatment can be conducted in the extraction plant. Furthermore, seaweed waste biomass is easy to hydrolyse due to its low lignin content (Uju et al., 2015) and this is more easily carried out than for terrestrial biomass, such as bagasse (Uju et al., 2016) due to the structure of seaweed cellulose having greater swelling and being more amorphous than bagasse (Roldán et al., 2017).

According to report delivered by Indonesian government, seaweed culture in Indonesia covered 267,800 ha. FAO reported that Indonesia produced 11,050,301 t of fresh seaweed, which was equal to 38% of world production and since 2008, Indonesia has been one of the largest seaweed producers (Valderrama et al., 2013). However, not all coastlines are suitable for seaweed culture, as the gel strength from agar is significantly affected by nutrient and environmental factors of the seawater, including the temperature, dissolved inorganic nitrogen and alkalinity (Chirapart et al., 2006).

Seaweed has a shorter life cycle than other terrestrial plants (Hal et al., 2014) and grows 3–4 times faster (Jung et al., 2013). For example, the productivity and growth rate of *Kappaphycus alvarezii* reached 50.9 g/m^2/d and 6.3%, respectively (Roldán et al., 2017). In addition, seaweeds can be cultured without additional fertilizer (Lee and Lee, 2016) and can be harvested up to five times annually (Goh and Lee, 2010). Therefore, seaweeds have been of great interest as sustainable feedstocks for biochemical products; Indonesia has more than 15 commercial agar processing plants producing annually approximately 8,000 t of agar product from about 70,000 t of red seaweed feedstocks (Meinita et al., 2017). The agar and carrageenan yields were 20–35% and 31–43%, respectively (Marinho-Soriano and Bourret, 2005).

Carrageenan and agar are traditionally used as food feedstocks such gelling, thickening agents and stability (Campo et al., 2009; Álvarez-Viñas et al., 2019). A study using nine different consumable microalgae reported that one microalgal species contained 33.83% of protein, while another species had carbohydrate and edible fiber contents of up to 67.84% and 34.29%, respectively (Setthamongkol et al., 2015). Studies conducted on *Caulerpa lentillifera* and *Ulva reticulate* indicated that *C. lentillifera* was rich in phosphorus, calcium, magnesium and copper, while *U. reticulata* was rich in potassium, manganese and ferrous and both seaweeds showed potential as healthy foods for human diets or as source of ingredients with high nutritional value (Ratana-arporn and Chirapart, 2006).

The use of seaweed and its wastes to produce ethanol still faces major technical and economic challenges. Research was conducted to determine the saccharification and fermentation efficiencies of seaweed solid waste hydrolysate by *S. cerevisiae* under anaerobic conditions (Martosuyono et al., 2016). Previous research on the optimization of pretreatment and saccharification produced a high reduced sugar content that was comparable with utilization of raw seaweed sugar as the substrate (Martosuyono et al., 2015). Cellulose conversion of seaweed waste biomass was in the range 77–100% (Uju et al., 2015) and the bioethanol produced was in the range 10.83–13.80 g/L (Meinita et al., 2019). Aside from the economic feasibility constraint, seaweed utilization as bioethanol feedstock also supports community preparedness toward natural disasters, as bioethanol production from seaweed as a gasoline substitute could underpin a self-sufficient energy program for a region that is vulnerable to natural disasters, such as Nusa Ceningan Bali (Poeloengasih et al., 2014).

The current work investigated the potency of seaweed waste from agar industries for bioethanol feedstock on a commercial scale. The simulation objective was to produce a glucose solution to combine with filter cake (FC) from an agar extraction plant to produce bioethanol. Three simulation scenarios were used based on the modified saccharification process: extractive hydrolysis, hydrolysis with prior drying and unseparated filter aid material (FAM). Laboratory experiments were conducted to calculate cellulose conversion and ethanol yield from each scenario. The simulations then conducted based on operation condition, conversion and yield that similar to laboratory scale at the basis calculation 500 L of fermentation tank.

Materials and Methods

Filter cake characterization

Two types of filter cake (FC) were obtained from two agar extraction plants. Plant No.1 was located in Banten province, Indonesia. Its agar powder production capacity was 150 t/mth (1,800 t/year). The agar powder was mostly produced from cultivated seaweed (*Gracilaria*) mixed with small amounts of wild-harvested *Gelidium* and *Pterocladia*. Plant No.2 was located in East Java, Indonesia and had a plant capacity of 20 t/mth with plans to expand there with a third plant.

The FC were composed of lignocellulosic biomass, agar and perlite. Perlite was added to process as filter aid material (FAM). All components were analyzed. Ash content analysis referred to the procedure of NREL/TP-510-42622, January 2008. Determination of lignin, hemicellulose and cellulose referred to the procedure NREL/TP-510-42618, revised August 2012. All required chemicals were analytical grade and all instruments used in the procedures were maintained using a laboratory that was ISO 17025 accredited.

Symbols and assumptions used in simulation scheme

The defined variable values for simulation are listed in Table 1. Equipment specifications were taken from commercial brochures and are listed in Table 2. There was no prior selection of best equipment performance among available producers. However, more specific requirements were applied to the dryer as it needed to be low-temperature to avoid gel formation within the FC by residual agar.

Table 1 Variable values, units and symbols used in simulations

Variables	Symbol	Unit	Notes
Initial water loading for extraction	A	g	Assumed for calculation
Initial filter cake loading for extraction	m^{fc}	g	
Extract from stage number (1,2,3,…n)	E_n	L	
Residue from stage number (1,2,3,…n)	R_n	g	
Number of extraction stage	n	-	
Cellulase enzyme loading	E	FPU/gbiomass	
Glucose concentration in extract	ω_g^{En}	g/L	
Cellulose conversion to glucose	X		
Glucose conversion to ethanol	Y		
Heat capacity of sugar solution 5%w (35°C)	Cp_{sugar}	kJ/kgK	4.07
Heat capacity of pure ethanol (67°C)	$Cp_{ethanol}$	kJ/kgK	3.06
Heat capacity of perlite (http://www.pullrhenen.nl)	$Cp_{pearlite}$	kJ/kgK	0.838

Table 2 List of equipment used in simulations

Equipment	Description	Power (kW)	Ref.
Filter press	Plate size 1,300×1,120 mm; number of plates 60; surface area 21. 2 m^2, cake max volume 284 L; inlet pump 2.46 L/min	1.1	Toro Equipment (2013)
Slurry pump	Pump capacity 20 L; pump head 30 m; outlet diameter 40 mm; 10 m^3/hr	3	SBMC (2018)
Agitation vessel	4 blades; no baffle; operating volume 100 mL	0.12	Bardant et al. (2017)
Low Temperature Filter Cake Drying Machine Heat Pump Dryer	Dehydration capacity 100 L; dimensions 4.75 m ×2.4 m ×2.78 m; moisture inlet 60–75%; moisture of dried material 10–50%; duration 1 hr; electric heater for air.	27.5	Kinkai (2008)

The transporting pump was considered the same for all three scenarios. Calculations for the energy required for ethanol purification were based on data from the existing ethanol production plant. The simulations used conventional ethanol distillation to the azeotrope point (95% v), which required 5,000 kJ/L of ethanol as the energy source. Adjustment was required when the glucose concentration was lower than 12% by weight. For dehydration via adsorption to purify alcohol above the azeotrope point, an additional 800 kJ/L were required to produce fuel grade ethanol (Austin, 1984).

Three simulation scenarios were studied. The process diagram for the enzymatic hydrolysis extraction scenario is shown in Fig. 1A. Basically, this process accumulated the glucose from biomass hydrolysis that was uniformly embedded with the FAM in the FC. The optimum number of extraction stages was obtained in this simulation. The second scenario applied a drying process prior to single stage hydrolysis, as shown in Fig. 1B. The third scenario carried the FAM all the way to distillation. This was the simplest approach compared to the previous two, as shown in Fig. 1C. The lignin and FAM in the filter cakes were assumed inert. The glucose solution was assumed to be homogenous. The initial water and FC loading were simulated based on calculations.

Calculation for simulation

The first enzyme that used in hydrolysis was cellulase. Two types of cellulase were used in the hydrolysis reaction. Cellulase I and cellulase II worked together to parse the 3D and fibrous cellulose structures and then to cut the cellulose polymers in half. Each extraction stage duration was customized for 8 hr. It was assumed that Cellulases I and II worked well for 8 hr and had completed the parsing of the attached cellulose. This structural change made the hydrolyzed cellulose more dilute and detached it from the particle surface of the FAM. The 8 hr assumption was reasonable based on qualitative observation on the mixture viscosity in the previous study in EFB pulp hydrolysis process (Bardant et al., 2017).

The second enzyme was β glucosidase, which converted cellobiose into glucose. The agitation was prolonged during the last stage to ensure sufficient time for the β glucosidase to act. At this stage, Cellulases I and II were still hydrolyzing the diluted cellulose microfiber into cellobiose. Adopting similar environmental conditions to those for EFB pulp hydrolysis, all extractive hydrolysis processes were simulated at pH 4.5–5 and at room temperature. Thus, no energy was required to maintain the process temperature.

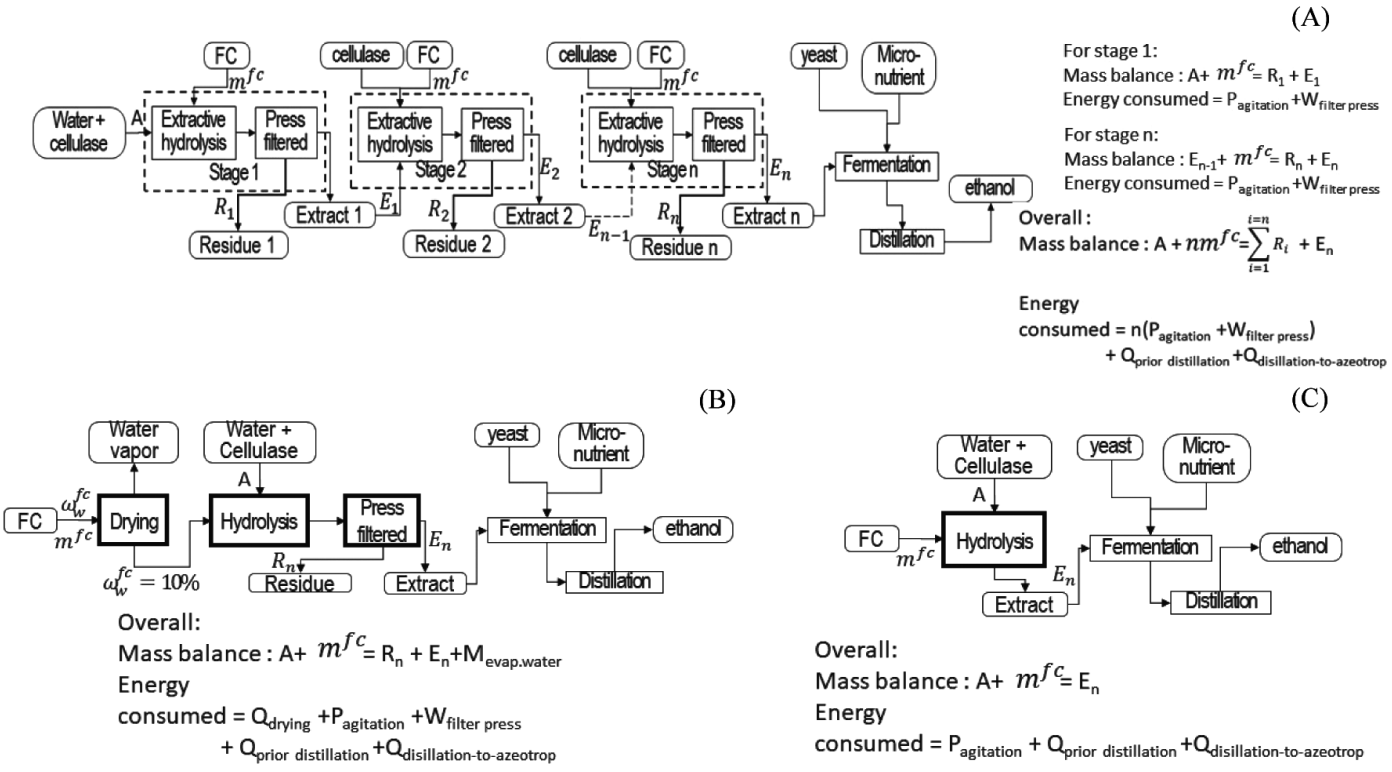

Fig. 1 Simulation diagram, related symbols and basic equation for mass and energy balance for: (A) enzymatic hydrolysis extraction of filter cake from agar extraction plant; (B) enzymatic hydrolysis with prior drying of filter cake from agar extraction plant; (C) unseparated filter aid material

Since the FAM and lignin were assumed inert and the saturated water fraction in the residue was the same for every stage of extraction, then:

$$m^{fc}\omega_z^{fc} = R_n\omega_z^r \quad (1)$$

$$m^{fc}\omega_L^{fc} = R_n\omega_L^r \quad (2)$$

$$\omega_w^R \cdot R_1 = \omega_w^R \cdot R_2 = \omega_w^R \cdot R_n = WR \quad (3)$$

Inserting Equation 1 and 2 into Equation 3 provides:

$$\begin{aligned} WR &= \omega_w^R \cdot m^{fc}\left(\omega_Z^{fc}+\omega_L^{fc}+(1\text{-}X)\omega_C^{fc}\right) \\ WR &= \omega_w^R \cdot m^{fc}\left(1\text{-}X\,\omega_C^{fc}\right) \\ WR &= B\cdot m^{fc}\ ;\ \text{with } B = \left(1\text{-}\,X\omega_C^{fc}\right)\omega_w^R \end{aligned} \quad (4)$$

The amount of extract by assuming the water density is equal to 1, the first stage of extraction can be calculated as:

$$\begin{aligned} &E_1 = A+\omega_W^{fc}\cdot m^{fc}\text{-}WR\text{-}0.1\omega_C^{fc}\cdot m^{fc}; \\ &\quad [0.1\omega_C^{fc}\cdot m^{fc} \text{ is water consumed by hydrolysis reaction}] \\ &E_1 = A+m^{fc}(\omega_W^{fc}\text{- B-}0,1X\omega_C^{fc}(1\text{-}\omega_w^R) = A+H\cdot m^{fc} \\ &H\ = (1.1\omega_w^R\omega_C^{fc}\text{-}0.1\omega_C^{fc}\,)X \\ &E_n = A+nH\cdot m^{fc} \end{aligned} \quad (5)$$

The glucose solution from hydrolysis was assumed homogenous; thus at the first stage:

$$\frac{m^{fc}\cdot\omega_C^{fc}\cdot X\cdot 1,1}{E_1+WR} = \omega_g^{E1}$$

$$\frac{D\cdot m^{fc}}{E_1+WR} = \omega_g^{E1};\ \text{With } D = \omega_w^R\cdot\omega_C^{fc}\cdot X\cdot 1,1$$

$$E_1\cdot\omega_g^{E1} = D\cdot m^{fc}\cdot\left(\frac{E_1}{WR+E_1}\right)$$

For the second stage:

$$\omega_g^{E2} = \frac{E_1\cdot\omega_g^{E1}+D\cdot m^{fc}}{E_2+WR}$$

Expanding the equation further, the glucose weight fraction in the extract of the n^{th} stage can be defined as:

$$\omega_g^{En} = \frac{E_{(n-1)}\cdot\omega_g^{E(n-1)}+D\cdot m^{fc}}{E_n+WR} \quad (6)$$

The iteration calculation was conducted by computing Equations 5 and 6 simultaneously using the Microsoft Excel software.

Overall, the mass balance for filter cake hydrolysis with prior drying can be calculated using:

$$\begin{aligned} E_n &= A+m^{fc}\text{-}R_n \\ E_n &= A+m^{fc}\text{-}\left[\frac{0,9m^{fc}\left(\omega_Z^{fc}+\omega_L^{fc}+\omega_c^{fc}(1\text{-}X)\right)}{(1\text{-}\omega_w^r)}\right] \end{aligned} \quad (7)$$

The moisture content in the dried filter cake was set at 10% based on information regarding the best dryer performance. The glucose concentration was calculated using:

$$\begin{aligned} \omega_g^{En} &= \frac{m^{fc}\cdot X\cdot\omega_c^{fc}\cdot\frac{nMrGlucose}{MrCellulose(n)}}{En} \\ \omega_g^{En} &= \frac{1.1\cdot m^{fc}\cdot X\cdot\omega_c^{fc}}{En} \end{aligned} \quad (8)$$

The amount of initial FC loading (m^{fc}) and the initial water loading for extraction were calculated based on iteration, where the goal was to use a amount of extract to the previous scenario to simplify the comparison. Water was added ionically as the cellulase solution in the hydrolysis process to overcome the technical problem of mixing the feed with the cellulase solution. Dried FC was added gradually to the cellulase solution while mixing. At the end of hydrolysis, some of the glucose was trapped in residue; this amount was calculated using Equation 4.

Equations 9 and 10 were used in the unseparated filter aid material scenario to calculate the amount of fermentation feed and its glucose concentration. The difference was E_n in slurry form.

$$E_n = A+m^{fc} \quad (9)$$

$$\omega_g^{En} = \frac{1.1\cdot m^{fc}\cdot X\cdot\omega_c^{fc}}{En} \quad (10)$$

Determination of conversion in laboratory-scale experiments

The equations containing conversion variables X were determined from the laboratory experiments. Some general operational conditions were kept constant in all three experiments. Enzyme solution addition was set at 20 FPU/g biomass and hydrolysis was conducted at pH 4.5–5. The mixture was stirred using a constant mixing power

at 12 W/100 g during the hydrolysis reaction. Fermentation was conducted by adding 1% by weight of powdered yeast produced (Le Satre, France), 0.3% urea and 0.1% NPK before storing in a dark room at 26–28°C for 72 hr. The urea and NPK were technical grade. Stirring was applied only during the first 15 min when the fermentation started, to ensure that the yeast and urea were diluted. The other operational conditions are described in Table 3.

In the first experiment to determine the cellulose conversion from enzymatic hydrolysis extraction, the FC sample was divided into five parts. For each part, the FC was added gradually into the enzyme solution and the hydrolysis reaction was allowed to occur for 8 hr. Then, the mixture was press-filtered and the filtrate used for the next hydrolysis extraction steps The FC was added in water to its saturation point to ensure the extract solution volume after press-filtering was similar with the initial volume of enzyme solution. Samples were taken from each extraction stage to determine the reducing sugar content.

The second experiment determined the cellulose conversion by hydrolysis with prior drying of the FC in an oven at 55°C for 16 hr before it was fed into the hydrolysis experiment. The FC was added gradually into the enzyme solution and then hydrolyzed for 48 hr. Then, the mixture was press-filtered and the extract used in the fermentation process. The extract was sampled to determine its reducing sugar content.

The third experiment determined the cellulose conversion using unseparated FAM with no special additional treatment. The FC was added gradually into the enzyme solution and the hydrolysis reaction was allowed to occur for 48 hr. Samples were taken to determine its reducing sugar content. Then, yeast, urea and NPK were added to the mixture, before storing for fermentation.

Samples of the fermentation products from all experiments were diluted twice and then distilled until the volume of the distillate equaled the initial volume. The density of each distillate was measured at 15°C to determine the ethanol concentration in the distillate as a volume percentage. The ethanol yield was calculated using:

$$\text{Ethanol yield} = \frac{V_{\text{fermentation broth}} \times C_{\text{ethanol in broth}}}{m_{\text{cellulose in FC feed}}} \quad (11)$$

Results and Discussion

Filter cake characterization

The results of FC characterization are shown in Fig. 2. The parameters required for the simulation are summarized in Table 4. Both large-scale commercial agar extraction plants used FAM in the pressed filter. The ratio of FAM added to process seaweed prior the filtration was confidential and beyond the scope of this study. FC samples from plants 1 and 2 were used in the laboratory-scale experiment to determine the conversion - and that was applied in the simulation of large-scale ethanol production.

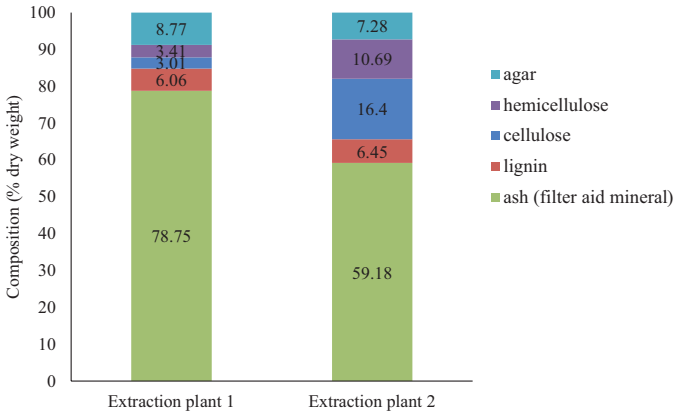

Fig. 2 Weight fraction of filter cake from agar extraction plant

Table 3 Operational conditions for laboratory experiment to determine cellulose conversion for three scenarios of ethanol production from filter cake (FC) from agar extraction plant

Simulation Scenario	FC substrate load (g)	Substrate component			Enzyme solution (mL)	Total working volume (mL)	Hydrolysis duration (hr)	Fermentation duration (hr)
		Cellulose (g)	FAM (g)	Water (g)				
Enzymatic hydrolysis extraction	From extraction plant 1				150	200	40 5 stage extraction, 8 hr each stage	72
	258*	1.7	44.61	202				
	From extraction plant 2							
	250*	10.3	37.5	188				
Hydrolysis with prior drying	50**	9.7	29.6	0.7	200	200	48	72
Unseparated FAM	50	6.56	20.1	13.5	87	100	48	72

* Filter cake substrate added with water to its saturation. Added at 50 g in each stage

** Filter cake substrate already dried to moisture content less than 10%

Table 4 Summary of measured parameters used in simulations

Variable	Symbol	Filter cake origin	
		Plant 1	Plant 2
Cellulose weight fraction in filter cake (% (g) dw)	ω_C^{fc}	3.01	16.4
Filter aid weight fraction in filter cake (% (g) dw)	ω_Z^{fc}	78.75	59.18
Lignin weight fraction in filter cake (% (g) dw)	ω_L^{fc}	6.06	6.45
Saturated water fraction in filter cake (% (g))	ω_W^{fc}	78.28	75.71
Saturated water fraction in residue (% (g))	ω_W^{r}	78.28	75.71

dw = dry weight

Determination of ethanol conversion in laboratory-scale experiments

Ethanol conversion and other observation results from the laboratory experiment are shown in Table 5. The best conversion (89.14%) was obtained from the hydrolysis with prior drying scenario, with the conversion for the other two production scenarios being similar at 77%. The conversion calculations were based on the reducing sugar concentration measured after the hydrolysis process, compared to the amount of cellulose fed into the hydrolysis. FAM separation removed the unconverted cellulose from the production system before fermentation. On the other hand, the unseparated FAM scenario - kept the unconverted cellulose in the process during fermentation. Thus, hydrolysis still occurred during fermentation, so-called simultaneous saccharification fermentation (Olofsson et al., 2008; Dahnum et al., 2015). This was the main reason for the similar level of ethanol from the unseparated FAM schenario being quite similar to the two other scenarios , even though the glucose concentration without FAM separation was at the lowest (3.22% dw).

The results from the laboratory experiments were consistent with several other studies that are listed in Table 6. Specifically, the ethanol concentrations from the current study were similar to the results obtained by Sudhakar et al. (2016) that are the highest on the list.

Table 5 Laboratory experiment results for observed ethanol production route from waste of agar extraction plant 2

Parameter	Unit	Ethanol production scenario		
		Enzymatic hydrolysis extraction	Hydrolysis with prior drying	Unseparated FAM
Quantitative measurement				
Filter cake substrate load	g	250*	50**	50
Substrate component load				
Cellulose	g	10.3	9.7	6.56
Filter aid mineral	g	37.5	29.6	20.1
water	g	188	0.7	13.5
Glucose concentration	% (by weight)	4.25	4.56	3.22
Cellulose conversion	%	77	89.14	77.07
Fermentation broth	mL	150	85	96
Ethanol concentration in broth	%v	2	2.5	2.29
Residue	g (wet basis)	260	115	n.d.
Ethanol yield	mL/g/cell	0.291	0.219	0.335
Qualitative observation***				
Hydrolysis product	Phase	Solution	Solution	Slurry
Heated hydrolysis product	Phase	Solution	Solution	Solid
Fermentation product	Phase	Solution	Solution	Slurry

FAM = filter aid material; n.d. = not determined

Volume of fermentation broth from each simulation = 100 mL

* = filter cake substrate added with water to saturation. Added at 50 g in each stage

** = filter cake substrate already dried to moisture content less than 10%

*** = room temperature (26–28°C)

Table 6 Summary of other studies using seaweed as bioethanol feedstock

Seaweed type	Biomass type	Microorganism	Country	Ethanol yield	Reference
Red seaweeds					
Gracilaria verrucosa	Residual agar pulp	*Saccharomyces cerevisiae* HAU strain	India	14.89 g/L (1.91 %v)	Adams et al. (2011)
E. cottonii	Seaweed solid wastes	Sacc. cerevisiae (YSC2, type II)	Malaysia	0.27 g/g (0.34 %v)	Tan and Lee (2014)
Gelidium latifolium	Waste from agar extraction	Saccharomyces cerevisiae	Indonesia	10.83±0.3 g/L (1.38 %v)	Meinita et (2017)
Brown seaweeds					
Undaria pinnatifida	Discarded as waste	*Pichia angophorae* KCTC 17574	Korea	9.42 g/L (1.207 %v)	Uju et al. (2015)
Green seaweeds					
Floating residue	Industrial spent	Saccharomyces cerevisiae	China	0.143 L/kg (14.3 %v)	Goh et al. (2010)
Gracilaria corticata	Extracted spent	Baker's yeast	India	3.02 g/L (0.38%v)	Sudhakar et al. (2016)
Alginate biomass	Extracted spent	Baker's yeast	India	1.61 g/L (2.06 %v)	Sudhakar et al. (2016)

%v = percentage by volume; g/g = gram per gram

There was an interesting qualitative observation on the appearance of the product from hydrolysis. FAM separation before fermentation produced a glucose solution product that had high value because the agar impurities in the extracts were not sufficient for conversion of the solution into gel after heating. In contrast, the solution turned into gel in the slurry hydrolysis product from unseparated FAM scenario.

Simulation results

The glucose concentration in the extract after each stage of extraction is shown in Fig. 4. According to this simulation, seven extraction stages were required to obtain additional glucose concentration of less than 1%. The increases in the glucose concentration at the seventh stage compared to the previous stage were 0.66% and 0.96% for the FC from extraction plants 1 and 2, respectively. The FC from agar extraction plant 1 with 3.01% cellulose content produced 1.17% glucose concentration in the final extract. This result was very close to the assumption of 100% conversion.

However, 4.25% glucose concentration was obtained in the final extract using hydrolysis with filter cake from extraction plant 2 with 16.40% cellulose content. This result was close to the simulation results with the assumption of 77% conversion. From the simulation, 5.76% glucose concentration would be obtained if enzymatic hydrolysis extraction of this FC could reach 100% conversion.

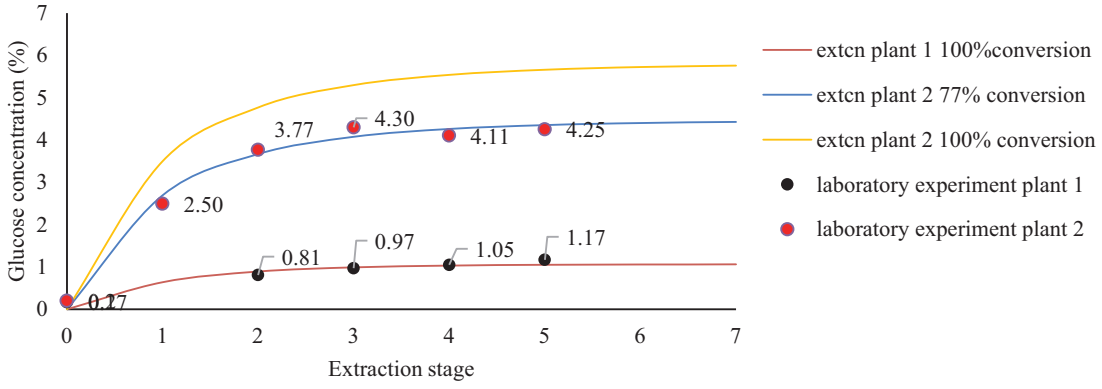

Fig. 3 Glucose concentration for each extraction stage in hydrolysis extraction process simulation compared to laboratory experimental results for filter cake from agar extraction plant 1 and agar extraction plant 2

Inserting ω_C^{fc}, ω_L^{fc}, ω_z^{fc} and ω_W^r obtained from laboratory measurements as shown in Table 4 for the FC from extraction plant No.2, then the following constants of B, H and D in Equation 4, 5 and 6 can be defined as

$$B = 0.7571–0.2602X; \quad H = 0.219X; \quad D = 0.0918X$$

Equation 5 can be written as:

$$En = A+0.2519X \cdot n \cdot m^{fc} \quad (12)$$

The value of conversion (X) was 0.77 as obtained from the laboratory experiment. This was used as the first basis for calculating the mass and energy balance for the first simulation scenario. Filter press capacity was defined as the bottleneck in the overall process in this first scenario. To run the filter plate at its highest energy efficiency, residue from each extraction stage was set at 284 L or 326 kg. Thus, the overall mass balance was calculated on this basis and is summarized in Table 7 for comparison with the other scenarios.

Simulation of filter cake hydrolysis with prior drying started by inserting values for ω_C^{fc}, ω_L^{fc}, ω_z^{fc} obtained from laboratory measurement for extraction plant 2, and ω_W^r resulted in 78% by weight. Then, Equation 7 then can be written as:

$$E_n = A–(4.09 – 1.38X)m^{fc} \quad (13)$$

$$\omega_g^{En} = \frac{0.3781 \cdot X}{\frac{A}{m_{fc}} - 4.09 + 1.38X} \quad (14)$$

The value of conversion (X) was 0.89 as obtained from the laboratory experiment. This was used as the first basis for calculating the mass and energy balance for the hydrolysis with prior drying simulation scenario. The capacity of the dryer was 100 L or 115 kg FC/hr and at its best performance it could reduce the moisture content to 10% by weight. For the 500 L extract volume as in the previous scenario, the glucose concentration obtained from the iteration was 2.40% by weight.

Simulation of the unseparated FAM started by inserting the conversion value (X) as 77.07% and ω_C^{fc} using Equation 10. The values for m^{fc} and A were simulated to obtain a value for E_n that was close to 500 L and with a similar glucose concentration to the laboratory results. It was found that the closest E_n value was 524 L, with the main electricity consumption (3,081.6 kWh) being for the agitation motor in hydrolysis.

Comparison of three simulation results and their further requirements for ethanol production

Comparison of the simulation results for the scenarios is shown in Table 7. In all cases, the main electricity consumption was for the agitation motor. The agitation was required to maintain good contact between the cellulase, biomass and water during the reactions (Rodmui et al., 2008). Thus, the requirements for maintaining the best enzymatic reaction rates, such as controlled agitation and temperature, were resource consuming. The simulations showed that the unseparated FAM scenario required the lowest electricity per liter of product.

The simulation indicated that extra heat and electricity were required for separating the FAM before fermentation. However, this opened the possibility of using glucose solutions for other

Table 7 Comparison of simulation results for three simulation scenarios

Parameter	Unit	Simulation		
		Hydrolysis extraction process	Hydrolysis with prior drying	Unseparated FAM
Product				
Volume extract	L	500	500	524
Glucose concentration	% (by weight)	4.25	4.56	3.22
Obtained ethanol 95%v	L	10	12.5	12.63
Requirement				
Filter cake	kg	2286.2	2861.8	475.4
Water	L	185.5	2158.8	50
Cellulase	L	12.05	53.28	4.06
Energy for hydrolysis	kWh	5475.5	16148.4	3081.6
Duration for hydrolysis	hr	72	48	48
Prior distillation	MJ	550.45	646.06	646.3
Distillation to azeotrope	MJ	833.33	1041.7	1052.3
Required heat	MJ/L	138.37	135.02	134.51
Required electricity	kWh/L	547.55	1291.87	244.872

purposes for financial gain. Such options could include high glucose syrup or as a sweetener for agar-derivative products, such as jelly and candy, where the glucose needed to be concentrated to suit the end product. Reverse osmosis filtration technology can be used for water removal from the glucose solution. Sterilization was required prior the filtration not only for food safety requirements but also to deactivate the cellulase, since most commercial reverse osmosis filters are made from cellulose acetate. The agar impurities need to be determined for this purpose to avoid unexpected gelation reaction.

FC hydrolysis of the feed to produce ethanol for use as an industrial solvent or fuel would not require FAM separation, as the FAM did not affect the yeast performance in producing ethanol. The ethanol could be used in the purification of other seaweed-based chemicals, such as carrageenan and alginate. This study has provided prospective insight for agar industrialists to expanded application to other seaweed-based chemicals.

Simulation the utilization of biomass from FC of agar extraction plant was conducted based on three scenarios: extractive hydrolysis, hydrolysis with prior drying and unseparated filter aid material. These simulations addressed the challenge of filter cake utilization as a substrate with a low biomass content. Only filter cake from extraction plant 2 with 16.4 % dw of cellulose was technically feasible based on the three simulation scenarios. The main electricity consumption for all three scenarios was by the agitation motor in the hydrolysis process. The unseparated filter aid scenario required the lowest amount of electricity per liter of product, since this scenario had the smallest agitation volume. The least heat requirement (134.51 MJ/L ethanol) was also for the unseparated filter aid scenario. However, this value was only 4 MJ/L less than the scenario of extractive hydrolysis that required the most heat. Further study is required to confirm this simulation result in a pilot plant experiment.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

This study was supported by a research program “Riset Scalling Up Produksi Bioethanol dari Limbah Pengolahan Agar” in the 2020 fiscal year in the Research and Development Center for Marine and Fisheries Product Processing and Biotechnology, Jakarta, Indonesia.

References

Adams, J.M.M., Toop, T.A., Donnison, I.S., Gallagher, J.A. 2011. Seasonal variation in *Laminaria digitata* and its impact on biochemical conversion routes to biofuels. Bioresour. Technol. 102: 9976–9984. doi.org/10.1016/j.biortech.2011.08.032

Álvarez-Viñas, M., Flórez-Fernández, N., Torres, M.D., Domínguez, H. 2019. Successful approaches for a red seaweed biorefinery. Mar. Drugs 17: 620. doi.org/10.3390/md17110620

Austin, G.T. 1984. Shreve’s Chemical Process Industries. McGraw-Hill Companies. Singapore.

Bardant, T.B., Susanto, H., Winarni, I. 2017. The RSM-CI emerging technology for enabling biochemical process: Ethanol production from palm plantation biomass waste in Indonesia. In: Haghi, A.K., Ribeiro, A.C.F., Pogliani, L., Balköse, D., Torrens, F., Mukbaniani, O.V. (Eds.). Applied Chemistry and Chemical Engineering, Vol 5. Apple Academic Press. Toronto, Canada, pp. 273–302.

Campo, V.L., Kawano, D.F., da-Silva. D.B., Carvalho, I. 2009. Carrageenans: Biological properties, chemical modifications and structural analysis - A review. Carbohyd. Polym. 77: 167–180. doi.org/10.1016/j.carbpol.2009.01.020

Chirapart, A., Munkit, J., Lewmanomont, K. 2006. Changes in yield and quality of agar from the agarophytes, *Gracilaria fisheri* and *G. tenuistipitata* var. *liui* cultivated in earthen ponds. Kasetsart J. (Nat. Sci.) 40: 529–540.

Dahnum, D, Tasum, S.O., Triwahyuni, E., Nurdin, M., Abimanyu, H. 2015. Comparison of SHF and SSF processes using enzyme and dry yeast for optimization of bioethanol production from empty fruit bunch. Energy Procedia 68: 107–116. doi.org/10.1016/j.egypro.2015.03.238

Goh, C.S., Lee, K.T. 2010. A visionary and conceptual macroalgae-based third-generation bioethanol (TGB) biorefinery in Sabah, Malaysia as an underlay for renewable and sustainable development. Renew. Sust. Energ. Rev. 14: 842–848. doi.org/10.1016/j.rser.2009.10.001

Goh, C.S, Tan, K.T., Lee, K.T., Bhatia, S. 2010. Bio-ethanol from lignocellulose: Status, perspectives and challenges in Malaysia. Bioresour. Technol. 101: 4834–4841. doi.org/10.1016/j.biortech.2009.08.080

Jung, K.A., Lim, S.-R., Kim, Y., Park, J.M. 2013. Potentials of macroalgae as feedstocks for biorefinery. Bioresour. Technol. 135: 182–190. doi.org/10.1016/j.biortech.2012.10.025

Kinkai. 2008. Kinkai Automatic Sludge Dryer. Commercial Brochure Ghuangzou Kaineng Electr. Equip. Company. Ghuangzou, China.

Kongkiattikajorn, J., Sornvoraweat, B. 2011. Comparative study of bioethanol production from cassava peels by monoculture and co-culture of yeast. Kasetsart J. (Nat. Sci.). 45: 268–274.

Lee, O.K., Lee, E.Y. 2016. Sustainable production of bioethanol from renewable brown algae biomass. Biomass Bioenergy. 92: 70–75. doi.org/10.1016/j.biombioe.2016.03.038

Li, H., Yu, X., Jin, Y., Zhang, W., Liu, Y. 2008. Development of an eco-friendly agar extraction technique from the red seaweed *Gracilaria lemaneiformis*. Bioresour. Technol. 99: 3301–3305. doi.org/10.1016/j.biortech.2007.07.002

Limtong, S., Deejing, S., Yongmanitchai, W., Santisopasri, W. 1998. Construction of high ethanol fermenting halotolerant hybrid by intergeneric protoplast fusion of *Saccharomyces cerevisiae* and *Zygosaccharomyces rouxii*. Kasetsart J. (Nat. Sci.). 32: 213–223.

Marinho-Soriano, E., Bourret, E. 2005. Polysaccharides from the red seaweed *Gracilaria dura* (Gracilariales, Rhodophyta). Bioresour. Technol. 96: 379–382. doi.org/10.1016/j.biortech.2004.04.012

Martosuyono, P., Hakim, A., Fawzya, Y.N. 2015. Chemical pretreatment and enzymatic saccharification of seaweed solid wastes. Squalen. Bull. Mar. & Fish. Postharvest & Biotech. 10: 61–71.

Martosuyono, P., Munifah, I., Ningrum, G.A. 2016. Ethanolic fermentation efficiency of seaweed solid waste hydrolysate by *Saccharomyces cerevisiae*. Squalen. Bull. Mar. & Fish. Postharvest & Biotech. 11: 7–12.

Meinita, M.D.N, Marhaeni, B., Jeong, G., Hong, Y. 2019. Sequential acid and enzymatic hydrolysis of carrageenan solid waste for bioethanol production: A biorefinery approach. J. Appl. Phycol. 31: 2507–2515. doi.org/10.1007/s10811-019-1755-8

Meinita, M.N.D., Meinita, Marhaeni, B., Hong, Y., Jeong, G. 2017. Enzymatic saccharification of waste from *Gracilaria verrucosa* and *Gelidium latifolium* for bioethanol production. J. Appl. Phycol. 29: 3201–3209. doi: 10.1007/s10811-017-1205-4

Niyomvong, N, Boondaeng, A. 2019. Ethanol production from cassava stem using *Saccharomyces cerevisiae*. TISTR 5339 through simultaneous saccharification and fermentation. Agr. Nat. Resour. 53: 667–673. doi.org/10.34044/j.anres.2019.53.6.15

Olofsson, K., Bertilsson, M., Lidén, G. 2008. A short review on SSF - process option for ethanol production from lignocellulosic. Biotechnol. Biofuels. 1: 7. doi: 10.1186/1754-6834-1-7

Pimpakan, P., Yongmanitchai, W., Limtong, S. 2012. Bioethanol production from sugar cane syrup by thermo-tolerant yeast, *Kluyveromyces marxianus* DMKU3-1042, using fed-batch and repeated-batch fermentation in a nonsterile system. Kasetsart J. (Nat. Sci.). 46: 582–591.

Poeloengasih, C.D., Bardant, T.B., Rosyida, V.T., Maryana, R., Wahono, S.K. 2014. Coastal community empowerment in processing *Kappaphycus alvarezii*: A case study in Ceningan Island, Bali, Indonesia. J. Appl. Phycol. 26: 1539–1546. doi.org/10.1007/s10811-013-0153-x

Ratana-arporn, P., Chirapart, A. 2006. Nutritional evaluation of tropical green seaweeds *Caulerpa lentillifera* and *Ulva reticulate*. Kasetsart J. (Nat. Sci.). 40(Suppl.): 75–83.

Roldán, I.U.M., Mitsuhara, A.T., Desajacomo, J.P.M., de Oliveira, L.E., Gelli, V.C., Monti, R., do Sacramento, L.V.S., Masarin, F. 2017. Chemical, structural, and ultrastructural analysis of waste from the carrageenan and sugar-bioethanol processes for future bioenergy generation. Biomass Bioenergy. 107: 233–243. doi.org/10.1016/j.biombioe.2017.10.008

Rodmui, A., Kongkiattikajorn, J., Dandusitapun, Y. 2008. Optimization of agitation conditions for maximum ethanol production. Kasetsart J. (Nat. Sci.). 42: 285–293.

Shuang Bao Machinery Company (SBMC). 2018. UHB-ZK Horizontal Slurry Pump. Commercial Brochure, ShuangBao Mach. Company. Shanghai, China.

Setthamongkol, P., Tunkijjanukij, S., Satapornvanit, K., Salaenoi, J. 2015. Growth and nutrients analysis in marine macroalgae. Kasetsart J. (Nat. Sci.). 49: 211–218.

Shet, V.B., Sanil, N., Bhat, M., Naik, M., et al. 2018. Acid hydrolysis optimization of cocoa pod shell using response surface methodology approach toward ethanol production. Agr. Nat. Resour. 52: 581–587. doi.org/10.1016/j.anres.2018.11.022

Sritrakul, N., Nitisinprasert, S., Keawsomponga, S. 2017. Evaluation of dilute acid pretreatment for bioethanol fermentation from sugarcane bagasse pith. Agr. Nat. Resour. 51: 512–519. doi.org/10.1016/j.anres.2017.12.006

Sudhakar, M.P., Merlyn, R., Arunkumar, K., Perumal, K. 2016. Characterization, pretreatment and saccharification of spent seaweed biomass for bioethanol production using baker's yeast. Biomass Bioenergy. 90: 148–154. doi.org/10.1016/j.biombioe.2016.03.031

Tan, I.S., Lee, K.T. 2014. Enzymatic hydrolysis and fermentation of seaweed solid wastes for bioethanol production : An optimization study. Energy 78: 53–62. doi.org/10.1016/j.energy.2014.04.080

Toro Equipment. 2013. Commercial Brochure. Draco Filter Press. Valladolid, Spain.

Uju, Wijiyanta, A.T., Goto, M., Kamiya, N. 2015. Great potency of seaweed waste biomass from the carrageenan industry for bioethanol production by peracetic acid-ionic liquid pretreatment. Biomass Bioenergy. 81: 63–69. doi.org/10.1016/j.biombioe.2015.05.023

Uju, Goto, M., Kamiya, N. 2016. Powerful peracetic acid-ionic liquid pretreatment process for chemical hydrolysis of lignocellulosic biomass. Bioresour. Technol. 214: 487–495. doi.org/10.1016/j.biortech.2016.04.121

Valderrama, D., Cai, J., Hishamunda, N., Ridler, N. 2013. Social and Economic Dimensions of Carrageenan Seaweed Farming: A Global Synthesis. Food and Agriculture Organization of the United Nations. Rome, Italy.

Hal, J.W.V., Huijgen, W.J.J., López-Contreras, A.M. 2014. Opportunities and challenges for seaweed in the biobased economy. Trends Biotechnol. 32: 231–233. doi.org/10.1016/j.tibtech.2014.02.007

Vaithanomsat, P., Apiwatanapiwat, W., Chumchuent, N., Kongtud, W., Sundhrarajun, S. 2011. The potential of coconut husk utilization for bioethanol production. Kasetsart J. (Nat. Sci.). 45: 159–164.

Wongsiridetchai, C., Chiangkham, W., Khlaihiran, N., Sawangwan, T., Wongwathanarat, P., Charoenrat, T., Chantorn, S., 2018. Alkaline pretreatment of spent coffee grounds for oligosaccharides production by mannanase from *Bacillus* sp. GA2. Agr. Nat. Resour. 52: 222–227. doi.org/10.1016/j.anres.2018.09.012