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Green Fractionation of 2G and 3G Feedstocks for Ethanol Production: Advances, Incentives and Barriers

Behzad Satari
University of Tehran

Amit Jaiswal
Technological University Dublin, amit.jaiswal@tudublin.ie

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Green fractionation of 2G and 3G feedstocks for ethanol production: advances, incentives and barriers
Behzad Satari1 and Amit K Jaiswal2,3

Efficient release of fermentable sugars from the complex biomass structure such as second-generation or third-generation feedstocks by an appropriate enzymatic hydrolysis needs a prior biomass fractionation. This process facilitates the exposure of more cellulose and hemicelluloses for enzymatic hydrolysis. This review focused on ‘green fractionation’ of biomass by applying the principles of green chemistry for bioethanol production. Besides, the recent technological achievements in applying these principles for the fractionation have been discussed. For green fractionation, energy delivery systems are referred to as microwave and ultrasound. Besides, green cellulose solvents, biomass-derived solvents, and supercritical carbon dioxide play an important role in green biomass fractionations. Furthermore, ball milling and biological treatment are significantly considered in this regard. These novel technologies are superior processes than conventional fractionation techniques in terms of energy and mostly environmental point of view.

Addresses
1 Department of Food Technology, College of Aburaihan, University of Tehran, Tehran, Iran
2 Environmental Sustainability and Health Institute, Technological University Dublin - City Campus, Grangegorman, Dublin 7, Ireland
3 School of Food Science and Environmental Health, College of Sciences and Health, Technological University Dublin - City Campus, Grangegorman, Dublin 7, Ireland

Corresponding author:
Jaiswal, Amit K (amit.jaiswal@TUDublin.ie, akjaiswal@outlook.com)

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Introduction
Ethanol produced by fermentation routes from sugar and starch-based feedstocks (1st generation feedstocks) currently dominates the liquid fuel market. Starch based substrates are first converted to simple sugars and the produced sugars are commercially fermented to ethanol by Saccharomyces cerevisiae. Fermentation broth typically contains 8–16% ethanol and can be concentrated via distillation and purified by dehydration for fuel-grade ethanol production [1,2]. The biggest challenge of using the first-generation feedstocks for ethanol production is their limited resources. Besides, using them for fuel applications is in the face of stiff competition from the food chains. Therefore, sustainable ethanol production in the future is feasible only when 2G and 3G feedstocks, which is lignocelluloses and algae respectively, are being used as the primary source of fermentable sugars [3].

Unlike first-generation feedstocks, lignocelluloses are abundant, cheap, and originated from waste streams. However, their bioconversion to ethanol with existing technology cannot economically compete with first-generation feedstocks and efforts are underway for its commercialization [4,5]. Polymers composed of C-5 and C-6 sugars, such as cellulose and hemicellulose, and a three-dimensional polymer, lignin, are entangled and make a recalcitrant structure which protects the plant against microbial degradation. In order to facilitate the hydrolytic release of fermentable sugars, a ‘biomass fractionation’ process is necessary to unfold the compact structure and make it amenable for hydrolysis. Biomass fractionation is referred to as ‘pretreatment’ for lignocelluloses while it does not necessarily lead to a fractionation but a decrease in ‘biomass recalcitrance’ [6].

Algae are marine fast-growing photosynthetic species and are categorized to macro-algae and micro-algae depending on their size. Unlike terrestrial plants, algae do not necessarily need freshwater and can grow in seawater as well. Macro-algae (seaweeds) are brown, red, and green algae, and typically contain 23.8–67% carbohydrates, 4.8–23% protein, 0.53–4.8% lipid, and 14–42% ash, with no/little lignin. Micro-algae are microscopic algae and because of high lipid content, they have been used for biodiesel production. However, in some species up to 70% carbohydrates (monosaccharides, oligosaccharides, and polysaccharides), are accumulated in their biomass, which makes them promising feedstocks for ethanol production. Micro-algae are first cultivated, and their biomass is harvested for biological ethanol production by the same process as the 2G feedstocks do. The fractionation process for the microalgal biomass is referred as cell wall disruption, which is performed in a relatively mild condition compared with lignocelluloses and macroalgae [2,7].

Numerous pretreatment methods for lignocellulosic materials are discussed in the literature and are broadly
divided into three main categories, ‘physical’, ‘chemical’, and ‘biological’ [2**,5,8]; some of them are applicable for microalgal cell disruption. Despite industrial attention and environmental friendliness of some of these pretreatment methods, most of them do not comply with the principles of green chemistry [5,9]. In this review article, we have tried to summarize major green fractionation methods currently in use. The objective of green fractionation is to reduce the usage of auxiliary chemicals, production of microbial toxic chemicals, and energy consumption. Figure 1 represents a microscopic schematic of green fractionation of biomass. Within the objectives of green fractionation, solvents play a determinant role and among them, those with low ESH (environmental, safety, and health) impacts are sought. Minimal use and/or replacement of environmentally harmful solvents and consumption of auxiliary chemicals such as surfactants, chelating agents are encouraged in the context of green fractionation [10,11**]. Microwave irradiation and ultrasound wave, with uniform energy delivery in an efficient way and short period of time, benefit the green fractionation from energy and environmental standpoints. More importantly, these techniques tend to intensify the fractionation process, leading to developing integrated biorefinery models. The superiority of green fractionating over conventional methods, recent technological development in applying green fractionation, barriers toward industrialization and possible solutions for overcoming them in industries are discussed in the later sections.

**Energy delivery systems for green fractionation of biomass: microwave and ultrasound**

In conventional heating, the direction of heat transfer from outside to the core of material makes a temperature gradient and takes long time for temperature to become uniform. In microwave (MW) processing, heat is generated within the product as a result of the transfer of electro-magnetic energy directly into the product. As a result of MW heating, the lignocellulosic components swell or fragment and therefore it becomes amenable for enzymatic hydrolysis [12]. MW treatment of microalgae makes their water content to reach its boiling point resulting in increasing the internal pressure and damaging the cell wall/membrane.

As one of the principles of green chemistry, unnecessary derivatization has to be avoided or minimized in chemical processes [11**]. Formation of inhibitory byproducts, for example, furan and lignin derivatives, which severely hamper the performance of hydrolytic enzymes and fermentable organisms, is minimal in MW based pretreatment of lignocellulosics compared with pretreatment using acid or base catalysts [12]. The negative impacts of the production of such byproducts are the consumption of additional raw materials and additional costs associated with detoxification of the pretreated substrates.

MW-assisted heating pretreatment can be used in combination with a solvent, water, or in a solvent-free

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**Figure 1**

Green fractionation using microwave or ultrasound (a) micro-algal sample and (b) lignocellulosic sample.
medium. Since MW interacts with dielectric water, high water content of biomass used, does not limit the application of this technology. In a solvent-free medium MW heating was reported to be so efficient and up to 64% hemicellulosic sugar recovery and enhanced glucose production by 70% in the enzymatic hydrolysis were achievable [12]. This is an indication of breaking covalent and hydrogen bonds in the lignocellulosic structure and confirms that the effect of MW heating does not limit to its heating effect. MW heating in combination with a catalyst or other pretreatment methods has synergistic effect on improving the enzymatic hydrolysis of pretreated lignocelluloses. Dilute acid or alkali [13,14], organic solvent [15,16], inorganic salts [17], and deep eutectic solvents and ionic liquids [18–20], were used with MW for pretreatment of lignocelluloses. MW-assisted dilute acid pretreatment of maize distillery stillage yielded up to 75.8% cellulose hydrolysis and produced low concentration of fermentation inhibitors for ethanol production [13]. Alio et al. [16] optimized the effects of process variables of a MW-assisted organosolv pretreatment of softwood species for obtaining a cellulose fraction with high yield and purity, recovering lignin, and producing the least amount of fermentation inhibitors. They obtained maximum cellulose yield of 82% with purity of 71% using MW heating at 175°C, ethanol–water (40:60) as solvent, and 0.25% sulfuric acid as catalyst. Similarly, Hernández et al. [15] obtained lignocellulosic fractions (lignin and hemicellulose), with preserved structure from agave bagasse in an MW-assisted ethanolosol process using a closed system (vacuum pressure) and an open system (atmospheric pressure). In this ethanolosol process, 0.1% HCl was also used as catalyst, which is usual in the processes using an organic solvent. In addition to the organic solvents, organic acids such as acetic acid and formic acid (with AlCl3, H2SO4, or HCl catalysts) have been also reported to extract tailor-made lignin from lignocellulosic waste in an MW-assisted process [21]. Notably, it is possible to depolymerize extracted lignin to monophenolic compounds such as syringol, vanillin, acetovanillone, and syringaldehyde, with high yield using oxidative reagents and MW-assisted heating (Panyadee et al. [14]).

Li et al. [22], in a review on microwave irradiation for pretreatment of lignocelluloses in 2016, mentioned rapid and uniform heating and thermal efficiency as the main advantages of MW heating compared with the conventional heating. However, despite technological development during the last 30 years, its industrialization has faced some obstacles, for example bioreactor design in scaled-up processes, lack of comprehensive understanding of the interaction among MW, biomass, and heating medium. Optimization of process variables such as mixing, pH, MW power and frequency, substrate loading and particle size and geometry and composition, viscosity, and downstream processing is not always straightforward to adapt from batch lab-scale to a bench scale and continuous industrial plant [23,24]. Besides, this is a biomass-dependent optimization approach and may vary depending on the type of biomass.

Later, in 2017, Kostas et al. [25] highlighted sustainability and energy efficiency of MW in the pretreatment of lignocelluloses; however, the abovementioned problem for industrialization still stated as their big concern. Besides the lack of existing knowledge on the biomass-MW interaction and high capital investment for transforming conventional heating by MW hinder the industrialization of this technology [26]. As a solution, making a comparison between the process in vessels using MW and conventional heating with analogous conditions was recommended.

Ultra-high frequency sound waves, by converting electric energy to mechanical energy and formation of local hot spots, can accelerate reactions at mild conditions [27]. The effects of ultrasonication on the structure of lignocelluloses were reported to be dewaxing, removing round shape silica bodies, reduction of particles size, increasing the surface area of pretreated biomass, losing or distracting the chemical linkages between the compounds in lignocelluloses, lowering the molecular weight of lignin and hemicelluloses, and changing or destroying the crystalline structure of cellulose [27]. Besides, ultrasound accelerates cellulose dissolution in solvents like ionic liquids. Muthuvelu et al. [28] confirmed the effectiveness of ultrasound-assisted alkali pretreatment of different lignocelluloses on bioconversion to ethanol. The authors applied a pretreatment at atmospheric conditions and notably, this technology produced fewer fermentation inhibitory residues.

Similarly, ultrasound has the ability to disrupt microalgal cell walls, thus releasing the carbohydrates and other microalgal derived constituents, for example, lipids [29]. Interestingly, disruption of microalgal cells in diluted media of large scales without harvesting and drying is possible via ultrasound. However, energy lost is high in this case since the size of cavitation is larger than most algal cells, which makes energy lost in the form of heat [30]. Similar to MW, ultrasonication is a nonselective process for microalgae treatment leading to no secondary pollution. Many microalgal-derived bioactives such as pigments and proteins, have applications in food industry. Customer demand and regulation prefer to use mild extraction processes to preserve the bioactivity of these sensitive molecules. Besides, high price of some of these bioactives is a major driving force for transforming to green fractionation for industries [31].

Another process with similar technology as ultrasound for biomass fractionation is hydrodynamic cavitation (HC).
In HC, due to large pressure difference in a moving fluid, micro-sized bubbles are formed and then the bubbles collapse leading to transformation of mechanical energy to kinetic energy and formation of hot spot shock waves [32]. As a result of these phenomena, in lignocellulloses, hydroxyl and superoxide radicals are generated in an aqueous neutral medium and hydrogen peroxide and hydroperoxy anion in alkali solutions which was reported to enhance the delignification process [33]. Sancheti and Gogate [34] reviewed the engineering aspects of chemical synthesis using ultrasound including bioreactor design, operating parameters, and some discussed reactions, are applicable to our discussion on pretreatment of lignocelluloses. Despite its effectiveness in biofuel production, a recent review highlighted the negative energy efficiency of ultrasounds in lab-scale studies [27].

In these processes, the co-production of valuable chemicals such as aromatic low molecular weight derivatives from lignin [35], essential oil, high-value pigments, proteins and other biologically active molecules, can improve the economy of ethanol production in an industrial process (Figure 2). Based on one of the principles of green chemistry [11**], this is important with regards to the reduction of a chemical waste production during the chemical processes. Lower costs for waste disposal in industrial processes are a major contributor to cost saving which is a major driving force for green fractionation to outpace the conventional fractionations. Improved employee health and safety and lower insurance premiums are also other possible contributing factors in cost saving for companies using these techniques. Indeed, at the end of the fermentation process byproducts separation is facilitated, and therefore a one-pot approach is performed. Besides, increasing consumer awareness toward renewable chemicals obtained via environmentally friendly techniques helps improving company reputation and socially attracts investors. The advantages and disadvantages of energy delivery for green fractionation are summarized in Table 1.

**The role of solvents in green fractionation**

**Green cellulose solvents**

Certain ionic liquids (ILs), concentrated phosphoric acid (CPA), and Deep eutectic solvents (DESs) are able to dissolve cellulose to some extent and this ability can be used for the pretreatment of lignocelluloses. The lignocellulosic components are partially dissolved in these solvents and can be then regenerated by adding an antisolvent (usually water) without major derivatization. The regenerated cellulose is much less crystalline than its original form and is highly reactive for enzymatic hydrolysis and conversion to ethanol [2**,36].

Favorable intrinsic properties such as low volatility, existing in liquid form at or below 100°C, high chemical and thermal stability, tailor-made properties, and high solvation power, make ILs as suitable solvents for biomass fractionation [37]. However, (eco)toxicity and low biodegradability limit their application from environmental point of view. The term ‘green solvent’ for ILs is referred to ILs produced in a ‘closed-loop’ biorefinery of lignocelluloses and is not included the petroleum-derived ILs [2**]. In addition, economy of solvent recycling is another determinant factor since IL recycling and purification is an energy-intensive step in biomass fractionation. Few studies in the literature are addressing these challenges in large

![Figure 2](https://example.com/f2.png)

**Figure 2**

Two routes for biological ethanol production from 2G and 3G feedstocks and recovery of valuable byproducts via a biorefinery standpoint.
scales with efficient IL recycling techniques and overcoming problems associated with phase separation [38,39].

DEs are liquid solvents formed from mixtures of Brønsted or Lewis acids and bases and contain hydrogen-bond acceptors and donors or (hydrated)metal salts. Natural DESs, composed entirely from plant metabolites such as glycerol, sugars, and natural acids, and are considered as ‘next generation solvents’ for various sustainable applications in chemical synthesis, extraction, and catalysis. DESs have lower cost than conventional ILs; however, the solubility of cellulose in DESs is lower than most ILs. Using DESs with high hydrogen-bond-accepting ability, for example, with Cl\(^-\), OAc\(^-\), and HCCO\(^-\), assisting ultrasound, and using surfactant, can enhance the cellulose dissolution in DESs [11*,40].

Current heating in ILs and DESs pretreatments is preferred to be replaced by MW-assisted heating. MW-assisted heating [TBA][OH] pretreatment was reported to deconstruct the lignin and hemicellulose structure of Eucalyptus, break the crystalline region, and make an eroded and pored microstructure, and sugar yield of up to 410.67 mg/g at 48 hour was obtained [19]. Only 45 s MW-assisted DES pretreatment at 800 W was reported to be highly effective in removing lignin and xylan of a variety of lignocelluloses and an increase by 2–5 folds in enzymatic hydrolysis was observed for the lignocellulosic biomass [20]. Another effect of coupling MW with DESs on lignocelluloses is cleavage of lignin–carbohydrate complexes and extraction of lignin oligomers, as stated by Liu et al. [18]. Similarly, sequential ultrasonication and DES was reported to be so effective pretreatment for oil palm fronds and resulted in significant lignin removal and xylose recovery [41].

**Table 1**

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<th>Fractionation method</th>
<th>Advantage</th>
<th>Disadvantage</th>
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| Microwave            | • Thermal efficiency  
                      | • Avoid unnecessary derivatization  
                      | • Rapid and uniform heating  
                      | • No temperature gradient in biomass  
                      | • Good interaction with water  
                      | • Facilitated by-product separation | • Difficulties in scaling up  
                      |                                                                 | • Lack of understanding of the interaction among MW, biomass, and heating media  
                      |                                                                 | • High capital investment |
| Ultrasound           | • Low level of waste generation  
                      | • Intensified processing  
                      | • Multiple products generation  
                      | • Efficient in disrupting micro-algal cell wall  
                      | • Applicable in diluted media of micro-algae  
                      | • Improved employee health and safety | • Energy lost in diluted media  
                      |                                                                 | • Difficulties in large-scale reactor design |

Biomass-derived solvents

Renewable solvents such as p-cymene, l-limonene, α-pinene, glycerol, gamma-valerolactone, organic acids, and furan derivatives, can be extracted and/or synthesized from plant biomass [42]. Mild pretreatment of lignocelluloses by cellulose-derived solvents, resulted in lignin fractionation/recovery and considerable increase in the following enzymatic hydrolysis [42]. Despite renewability, these solvents do not necessarily have low health or environmental impacts, and some are not categorized as green solvent. More importantly, economic sustainability should be wisely considered for industrial-scale application. Amongst them, gamma-valerolactone (GVL) has received great attention in the past few years for the deconstruction of lignocelluloses [43]. Pretreatment of lignocelluloses by GVL is performed in a concentrated GVL solution (>70% in water) at moderate temperatures of <140°C catalyzed by a dilute acid [43,44]. Despite many advantages, GVL has a high vapor pressure (10 Pa) and its high production costs limit its industrial applications [37].

Supercritical carbon dioxide

Supercritical carbon dioxide (sCO\(_2\)) was first utilized as an extracting solvent. It is a non-toxic, non-flammable, easily available, and low-cost solvent. Carbon dioxide above its critical temperature (31.1°C) and critical pressure (72.9 atm) behaves as a supercritical fluid, displaying the advantages of a gas (high diffusivity and low viscosity), and a liquid (high density and high solvation power). Chemical waste production is prevented in sCO\(_2\) pretreatment and the solvent can be easily washed away from raw materials by a simple depressurization process [45,46]. This is of great importance from the energy point of view since separation process is one of the most energy-intensive steps in the fractionation processes.

The water content of lignocelluloses was reported to have a determinant factor in the effectiveness of sCO\(_2\) pretreatment. Pretreatment of dry corn stover and switchgrass using sCO\(_2\) at 100–150°C and 3200–3500 psi for 1 hour resulted in a slight improvement in glucose yield.
[47]. On the other hand, pretreatment of agricultural residues with moisture content of 75% using sCO₂ (50–80°C, 17.5–25.0 MPa, 12–60 hour) resulted in threefold to fourfold increase in enzymatic hydrolysis yield than the raw materials [48]. Carbonic acid formation, biomass swelling, and hydrothermal-enhanced pretreatment (at temperatures above 100°C) were reported to be the positive effects of biomass moisture on the sCO₂ pretreatment. For microalgae, high-value carotenoids, pigments, and essential oil were reported to extract from biomass by sCO₂ [49]. A summary of advantages and disadvantages of main solvents applied in green fractionation are summarized in Table 2.

However, high energy requirement for compression of carbon dioxide to its supercritical pressure and high capital installation limit the commercial application of sCO₂ for a green fractionation. Besides, the application of sCO₂ in biomass fractionation is limited due to its zero dipole and low solvation capacity. Therefore, for extraction purposes, the solvation power of sCO₂ is improved by adding a polar co-solvent like ethanol or water; but, limited knowledge is available in the literature in this regard [50*].

Mild fractionation techniques

Ball milling

Ball milling or grinding is considered as an effective physical pretreatment of lignocelluloses and its first and foremost effects are the reduction of particle size and partial disruption of cellulose hydrogen-bonding network of lignocelluloses [51]. Ball milling pretreatment was reported to liberate (hemi)celluloses from Miscanthus and reduce the size of biomass to 30 μm without considerable affecting the cellulose crystallinity [52]. Besides, the accessibility of cellulose and hydrophilic capacity in the pretreated biomass were enhanced. On the other hand, ball milling made a reduction in crystallinity index of cellulose for corn stover [51]. Depending on the type of feedstock and final particle size, this process is usually considered as an energy-intensive pretreatment and acceptable for large-scale applications only in some cases.

An approach, referred to as ‘mechano catalysis’ or ‘mixmilling’ in the literature [53], is advantageous in terms of high performance and low chemical usage. The saccharification yield of bagasse and Pennisetum was considerably improved by this approach and lower amount of dilute alkali, compare to traditional biomass pretreatment, was consumed [54]. Similarly, a mechano-bio catalytic one-pot process was reported to be so efficient in obtaining high sugars titer and conversion from various lignocelluloses [55].

Ball milling is efficient in disintegrating the cell wall of microalgae, as well. Over 97% of cell disintegration for microalgae Chlorella vulgaris was achieved using bead milling and 85% lower energy was consumed at process time of 1 min [56]. Suarez García et al. [57] developed and validated a kinetic model for microalgal cell disintegration via bead milling and further concluded that a high bead filling, that is, >65%, is required in upscaled processes to ensure an energy-efficient process.

Biological fractionation

The use of biocatalysts is a green and sustainable technology attributing to the metrics of green fractionation [58]. Hydrolytic enzymes such as peroxidases, laccases, cellulases, and hemicellulases, can hydrolyze the lignocelluloses’ components or lancinate microalgal cell wall [1]. Some fungal species belonging to ascomycetes, basidiomycetes (including white-rot fungi and brown-rot fungi), and few anaerobic species were reported to secret extracellular hydrolytic and ligninolytic enzymes. While pretreatment with fungi generally takes too long, only few days/hours are enough for some bacterial pretreatment

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<td>Summary of advantages and disadvantages of green solvents for green fractionation of biomass</td>
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A bioprocess approach, called consolidated bioprocessing (CBP), integrates fractionation, enzymatic hydrolysis, and ethanol production. Some mucoralean fungi, for example, *Mucor indicus*; bacterial species from the genera *Clostridium*, and *Basidionymyces*; and yeast species of *Kluyveromyces, Clavispora*, and *Gryphicus*; were reported to have CBP ability for ethanol production [1]. This is a ‘one-pot’ synthesis approach and is advantageous in terms of reducing solvent usage and separation aids.

Zabed *et al.* [59] recently published a review on the biological pretreatment of lignocellulosic biomass and microalgae. Low energy consumption, mild operating conditions, low byproducts formation, and high selectivity are the main advantages of using enzymes in biomass fractionation. On the other hand, in industrial processes, this technology suffers from long reaction time and sometimes low efficiency, and also sugar consumption. Besides, obtaining competitive prices in fermentative production of enzymes needs further advancement in metabolic engineering for enhanced yield, production rate, and ease of purification. Moreover, despite using pure microbial cultures, variation in composition and structure of biomass makes this process a biomass-dependent technology.

**Conclusions**

The ‘green fractionation’ techniques would outpace the conventional fractionation and regulation, customer demand, and environmental benefits, are the major incentives for this transformation. Despite outstanding technological achievements in this area, adopting the green fractionation to the industries has faced some challenges that stem from lack of knowledge in lab-scale studies to bioreactor design for commercial applications. Despite high capital investment and operating costs for implementing some of these techniques, the revenues from high-value byproducts obtained via integrated biorefinery approaches would improve the economy of the fractionation processes.

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**Conflict of interest statement**

Nothing declared.

**References and recommended reading**

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


A highly cited article on the emerging technologies for the fractionation of lignocellulosic biomass.


In this review, the benefits of green chemistry (12 principles) by robust examples and by outlining the knowledge and future challenges were explored.


In this paper extraction of carbohydrates and protein was performed by ultrasound-assisted extraction and a comparison were made with pulsed electric field.


41. Ong VZ, Wu TY, Lee CBTL, Cheong NWR, Shakk KPY: Sequential ultrasonication and deep eutectic solvent pretreatment to remove lignin and recover xylose from oil palm fronds. Ultrason Sonochem 2019, 58:104038.


In this study, supercritical carbon dioxide ethanol co-solvent was used in order to extract Astaxanthin and lutein, which are two antioxidant used in nutraceutics and cosmetics.


This paper is a comprehensive review on biological pretreatment of lignocellulosic biomass and microalgae, and reported an increase by 22–159% in biofuel production from microalgae as the results of bacterial pretreatment.