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Review

Ultrasound-enhanced conversion of biomass to biofuels

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ABSTRACT

Two important challenges need to be addressed to realize a practical biorefinery for the conversion of biomass to fuels and chemicals: (i) effective methods for the degradation and fractionation of lignocelluloses and (ii) efficient and robust chemical methods for the conversion of bio-feeds to target products *via* highly selective catalytic reactions. Ultrasonic energy promotes the pretreatment and conversion process through its special cavitational effects. In this review, recent progress and methods for combining and integrating sonication into biomass pretreatment and conversion for fuels and chemicals are critically assessed. Ultrasonic energy combined with proper solvents allows destruction of the recalcitrant lignocellulosic structure, fractionation of biomass components, and then assists many thermochemical and biochemical reactions, with increased equilibrium yields of sugars, bio-ethanol and gas products by 10–300%. Sonication promotes hydrolysis, esterification and transesterification in biodiesel synthesis and leads to reduced reaction time by 50–80%, lower reaction temperature, less amounts of solvent and catalyst than comparable unsonicated reaction systems. For algal biomass, sonication benefits the disruption, lysis and content release of macro and microalgae cells, and reduces the time required for subsequent extraction and chemical/biochemical reactions, with efficiencies typically being improved by 120–200%. High-frequency ultrasound of 1–3 MHz allows harvesting of microalgae, liquid product separation and *in-situ* process monitoring of biomass reactions, while high-intensity ultrasound at 20–50 kHz activates heterogeneous and enzymatic catalysis of the biomass reactions. The use of ultrasound in conversion of biomass to biofuels provides a positive process benefit.

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1. Introduction

The lack of methods to circulate carbon sustainably is thought to be one of the dominant reasons for deterioration of our environment [1]. Techniques that efficiently circulate renewable carbon are an urgent priority and if the use of fuel-based devices is continued, efficient methods to produce biofuels are needed. Available biomass resources that can be considered for use as biofuels are mainly sugars, polysaccharides, lignocelluloses, chitosan, lipids, algae and polyols [2]. Some of the possible conversion paths of these materials to biofuels [2–7] are acid/base/enzyme-catalyzed hydrolysis of biomass to fermentable sugars, fermentation of cellulosic biomass and sugars to bio-ethanol, bio-hydrogen and other small molecules, catalytic hydrocracking, hydrogenation and reforming of lignocellulose to polyols, catalytic oxidation of lignocellulose to aromatic aldehydes, thermal gasification of cellulosic biomass to hydrogen and methane, and chemical/enzymatic esterification/transesterification of lipids to monoesters.

In the conversion of biomass resources to biofuels, three challenges must be met. The first challenge is the recalcitrance of lignocellulose [8] and the reticular connection of lignin [9]. Crude lignin–carbohydrate requires harsh conditions and specialized solvents to degrade, which makes processing complicated, environmentally problematic and economically unsustainable [3].

The second challenge is the uncertain chemical constituents contained in biomass. Raw biomass from different sources has varied contents of cellulose, hemicellulose, lignin, free sugars, wax, proteins, alkaloids, trace organic compounds, and inorganics [10].

The third and the most important challenge is the heterogeneity of biomass reaction systems. Biomass materials have low thermal conductivity that creates a barrier for heat and mass transfer [11]. Conversion and product selectivity in biomass systems tend to be poor due to insufficient catalyst–reactant contact. Immiscible alcohol–lipid–catalyst systems are a fundamental problem in biodiesel synthesis [12].

Ultrasonic energy provides a special physicochemical environment for processing biomass systems [13]. The high-energy impact and corrosion by high-intensity ultrasound to biomass system contributes to easier pretreatment, fractionation and chemical reactions of biomass materials under mild conditions, and results in increased reaction efficiency and higher catalytic activity over thermochemical methods (Challenges 1 and 2). The application of ultrasound energy intensifies mass and heat transfer in reactions, enhances the contact and disengagement of heterogeneous reactants, intermediates and products, and therefore accelerates the reaction rate or changes the kinetics (Challenge 3).

Reviews on ultrasonic applications are related to biomass treatment, such as sono-assisted lignocellulosic pretreatment [14,15], extraction of natural products [16], sonochemistry of carbohydrate compounds [17], catalytic esterification and transesterification of lipids [18–20], food processing [21], pretreatment and fermentation of organic wastes (e.g., bio-sludge) to gaseous products (e.g., H_2, CH_4) [22,23] and biochemical engineering/biotechnologies [24–26] such as biological wastewater treatment and bioremediation. However, critical assessment of ultrasound-assisted pretreatments and reactions of biomass for producing biofuels is needed to consider the role and mechanism of ultrasonic intensification and how it can be used advantageously in future biorefineries.

This review examines ultrasound-assisted pretreatments and reactions of biomass materials. Recent applications of ultrasonic energy in the pretreatment and conversion of lignocelluloses, biodiesel and microalgae are described and critically evaluated. Then, the potential of ultrasound for other related biomass processing (emulsification, demulsification and aggregation) and *in-situ* process monitoring are introduced and discussed. Finally, key issues are discussed and analyzed.

2. Physical mechanisms for ultrasonic energy

2.1. Ultrasound, energy transformation and acoustic cavitation

Ultrasound is mechanical acoustic wave with the frequency range from roughly 10 kHz to 20 MHz [27]. It imparts high energy to reaction medium by cavitation and secondary effects [13,28]. In a typical dynamic process of cavitational bubbles, numerous microbubbles containing solvent vapors are generated that grow and undergo radial motion as acoustic energy propagates through the liquid medium [29]. These microbubbles grow to a maximum of about 4–300 μm in diameter [29], and can be stable or transient. With low acoustic intensity, the radii of microbubbles periodically and repetitively expand and shrink (radial oscillation) within several acoustic cycles. While acoustic energy has sufficient intensity, some microbubbles are unstable within only one or two acoustic cycles. When the resonant frequency of bubbles exceeds that of ultrasonic field, the bubbles collapse within several nanoseconds, which creates special physical and chemical effects, and enhances thermochemical/biochemical reactions or treatment [29].

The unsymmetrical collapse of bubbles at a broad solid/solvent interface ($>$200 μm) produces **microjets** at high speed ($>$100 m/s) toward solid surfaces [27,30]. The instantaneous collapse of bubbles also produces strong **shockwaves** that might be up to 10^3 MPa [30,31]. This violent movement of fluid toward or away from the cavitational bubbles is defined as **micro-convection**, which intensifies the transport of fluids and solid particles and results in forces that can cause emulsification or dispersion depending on the conditions, while the strong shockwaves and microjets generate extremely strong shear forces over those of conventional mechanical methods, and are able to scatter liquid into tiny droplets or crush solid particles into fine powders.

The chemical effect of ultrasonic comes from **local hotspots** produced by cavitation. At the moment of bubble collapse, a huge amount of energy is released that cannot be immediately transferred to the surroundings. As a result, local hotspots are developed that have extremely high temperatures (ca. 5000 °C), high pressures (ca. 50 MPa) and high rates of heating and cooling in the bubbles ($>10^9$ °C/s) [27]. The extremely high temperature and pressure can destroy the crystalline state of solid materials, cause solids to melt or fuse solid particles when they collide with each other [30]. Ultrasonic energy can cause the formation of short-lifetime reactive radicals such as H• and HO• from reactants or solvent molecules at the moment of bubble collapse [27].

One of the most important aspects of applying ultrasonic energy to systems is how its energy is transferred to reactant solutions, which has three steps: 1) the transformation of electrical input to mechanical energy through a piezoelectric or piezomagnetic transducer; 2) the delivery of vibrational energy (acoustic energy) from the emission tip of the transducer to the liquid medium; 3) the conversion of the energy of ultrasonic streaming to the energy that activates reactants by acoustic cavitation [32]. Energy losses in any step will influence the total energy efficiency.

Direction-selective delivery of acoustic energy and its attenuation in homogeneous or heterogeneous system results in a nonuniform energy field. Acoustic cavitation efficiency, namely the conversion efficiency of ultrasonic energy to cavitational energy, also plays a role in mechanisms. Only cavitational bubbles that grow to a certain size can accumulate sufficient energy for reactant activation, since this energy is rapidly released with the radial motion of the bubble or as bubble collapse in transient cavitation. Therefore, acoustic cavitation is regarded as the most important mechanism in interpreting the effect of ultrasonic energy on reactions.

Many interesting studies have been reported on the description and evaluation of cavitation behavior in sonochemical reactors qualitatively and quantitatively, both with theoretical and experimental methods [29,33,34]. Through theoretical calculations at the thermodynamic and kinetic level, it is possible to estimate the growth, size, pressure, temperature and lifetime of cavitational bubbles, which are related to the energy level of bubbles [29]. Through computer simulation, it is possible to estimate the distribution of acoustic energy in an ultrasonic reactor with different geometries and operating parameters [33].

Various methods can be introduced to experimentally determine the velocity, size and concentration of cavitational bubbles in the reactor. The sudden change of acoustic pressure in sound field is regarded as the characteristic of cavitation, while the feature distribution in acoustic emission spectrum, namely the generation of subharmonic, ultraharmonic, or harmonic frequency, is used to discriminate between transient cavitation from stable cavitation [32,35]. Cavitational intensity is related to the population density of active cavitational bubbles and the released energy intensity in bubble collapse, and is normally quantified through the determination of secondary effects. Chemical methods help in the mapping of cavitational intensity distribution and active radical formation in the sonochemical reactors [36,37], including iodine method ($H_2O \rightarrow {}^\bullet OH + {}^\bullet H$, $I^- + {}^\bullet OH \rightarrow I_2^- \rightarrow I_3^-$), Fricke method ($H_2O \rightarrow {}^\bullet OH + {}^\bullet H$, $Fe^{2+} + {}^\bullet OH \rightarrow Fe^{3+} + OH^-$), salicylic acid method (the degree of salicylic acid hydroxylation under sonication), terephthalic acid method (terephthalic acid → hydroxyterephthalic acid), sonoluminescence (instantaneous flash during the collapse of cavitational bubbles), sonochemiluminescence (the reaction of cavitation-induced radicals with chemical reagents such as luminol), aluminum foil erosion and electrochemical redox process.

However, these analytical methods have their limitations. Chemical methods involving radical determination depend on the number of radical release at the collapse of cavitational bubbles, while aluminum foil erosion is more suitable for quantitative analysis of the intensity of secondary effects such as shockwave and microjets. Chemiluminescence relates the behavior of bubbles that achieve a certain energy level (bubble radius of 2.6–4.1 μm at 575 kHz), while sonoluminescence gives the most violent characteristics in bubble dynamics (active bubble radius of 5.4–5.5 μm at 575 kHz) [29]. Methods that use advanced technologies such as laser diffraction, pulsed multibubble sonoluminescence, laser phase-Doppler [38], methyl radical recombination and others [29,33] are used not only for analysis of single cavitational bubbles, but also for the acquisition of qualitative and quantitative information of bubble clusters or streamers [29].

Cavitation is the main mechanism for ultrasound intensification. The key to efficient application of ultrasound is control and selection of the energy intensity and population of active cavitation. The energy intensity of cavitation depends on the mean behavior of bubbles, while the population of active cavitation determines the cavitational efficiency. Many experimental methods have been developed to measure the population and secondary effects of cavitation, however, these methods have their limitations. Therefore, it is necessary to use theoretical method to estimate the energy level of cavitational bubbles.

2.2. *Basis for reactor design and parameters*

For processing of biomass materials with ultrasound, the selection of ultrasonic parameters such as ultrasonic mode (continuous or pulse), frequency, power, processing temperature, solvent, aeration and the design of reactors with proper geometric construction determines the level and distribution of energy intensity in the system, and thus influence efficiency and reliability of the results [13,39]. Depending on experimental objectives, parameter optimization can be very different. Nevertheless, the selection of ultrasonic reactors and parameters can benefit from many studies on bubble cavitation characteristics, including growth/radial oscillation/collapse, population/density, intensity and lifetime, as well as secondary effects such as shockwaves and micro-convection.

2.2.1. *Design of ultrasonic reactors*

In the design of ultrasonic reactors, some of the design parameters to be considered are reactor types (e.g., bath, probe, flat plate

Table 1
Common ultrasonic reactor types.

Reactors	Brief description	Frequency and power/intensity	Advantages	Disadvantages	Typical application cases	Scale-up
Liquid whistle	Ultrasound generated by mechanical oscillation	5–30 kHz [13], 1.5–2.5 W/cm^2	1) Low cost; 2) Suitable for continuous flow reactions	Low frequency and power output	1) Vegetable oil emulsification [44,45]; 2) Liquid–solid mixture homogenization [13]; 3) Animal fat hydrolysis [46]; 4) Industrial wastewater degradation [47]	Yes [41]
Ultrasonic bath	Transducer at bottom or side position of bath; reaction vessel fixed at some positions in bath	15 kHz–>1 MHz, Normally 1–2 W/cm^2	1) Low cost; 2) Commercialized	1) Need additional mechanical stirring; 2) Dispersed and inhomogeneous acoustic intensity distribution; 3) Low cavitational efficiency.	1) Cleaning of metal piece; 2) Simple chemical reactions	Possible
Probe reactor	Directly deliver of ultrasonic energy to liquid reactant through immersible horn	Up to >100 W/cm^2	1) High power output; 2) Concentrated energy deliver; 3) Commercialized	1) Easy erosion of horn tip [13]; 2) Concentrated and inhomogeneous acoustic intensity distribution; 3) Difficult control of reaction temperature; 4) Low cavitational efficiency.	1) Intensive chemical reactions; 2) Stubborn solid crushing.	Possible
Cup-horn reactor	Transducer fitted with a cup; the cup being the reactor [48–50]	20–504 kHz, 19–270 W[a] [48,50]	1) Concentrated energy deliver; 2) Avoid contamination of reactant from horn tip erosion	1) Only suitable for small quantity reactions	Vegetable oil saponification [49]	No
Resonating tubular reactor (Sonitube)	Ultrasound generated by axial vibration of stainless steel resonating tubes containing flowing reactant	20–35 kHz, Up to 2 kW[a] [48]	1) Avoid possible contamination of reactants; 2) Capable for handling large-scale feedstocks.	–	Phenol oxidation [52]	Possible [40]
Reverberative flow reactor	Intensified acoustic intensity by reflection and reverberation [54]	17–45 kHz, Up to 3 W/cm^2 [54]	Concentrated and intensified energy deliver, uniform ultrasonic field	–	Biodiesel synthesis [54]	Yes
Polygonal Reactor	Transducer array surrounds the polygonal vessel containing liquid reactant	–	Concentrated and intensified energy deliver, uniform ultrasonic field	–	Cellulose enzymatic hydrolysis [55,56]	Yes

[a] Values given for acoustic energy used are power ratings.

or tube), reactor geometry, transducer design and arrangement, and volume or scale of feedstocks for practical systems.

Table 1 lists and compares sonochemical reactors that are commonly used in ultrasound-assisted biomass reactions [13,40–56]. Commercialized bath and probe reactors are commonly used in laboratories, although they have an inhomogeneous distribution of acoustic field and low energy efficiency [33]. For example, probe-type reactors have the highest cavitational intensity within a distance of one wavelength (about 3–5 cm) from the irradiating surface of the transducer or horn tip. As a result, the greatest benefit of physical or chemical processing such as emulsification is realized within the distance of one wavelength for probe-type reactors [34,57].

For large-scale or commercial reactors, designs that use probes or transducers have to consider reactor geometry to be able to handle high and concentrated acoustic intensities [53,55,56]. Key points in reactor design and arrangement of ultrasonic probes and transducers are:

1) Position of ultrasonic horn in a sonochemical reactor. For a horn-type ultrasonic device, the position of the ultrasonic horn in the reaction vessel influences the feature of acoustic streaming and thus the distribution of cavitational activities [58]. Appropriate horn position depends on the reactor size, processing scale and even the height of liquid reactants [59]. Conventional ultrasonic horns are vertically dipped into the liquid solution to a depth of only several centimeters, which provides a poor streaming of acoustic energy in large-scale reactors. Acoustic velocity in the radial direction is only 10% of the vertical velocity [58]. The cavitational activity is limited to a very small zone near the ultrasonic horn that is only 10% for a 2.5 L reactor as an example [34]. Kumar et al. [34,60] proposed the horn to be horizontally immersed into the sonochemical reactor and longitudinally vibrated, with the immersion length of the horn to be 82% of the horizontal length of the reaction vessel based on a 7 L volume. This modification changes the acoustic streaming flow characteristics in the reactor, and it gives good power dissipation over the reactor volume, and thus generates a nearly uniform cavitational activity compared to conventional designs.
2) Surface and shape of the tip of the transducer or horn. Large tip diameters of the ultrasonic horn give low ultrasonic energy densities due to the large emitter surfaces, however, rapid deactivation of α-amylase was observed for large tip diameters from 1 to 7 mm [61]. Kadkhodaee and Povey [61] interpreted this unusual phenomenon occurred due to three reasons: i) reaction between •OH radicals and enzymes not taking place inside of the bubbles; ii) high densities of cavitational bubbles around 1 mm sonotrode tip causing serious acoustic attenuation; iii) high energy in individual cavitational bubbles being released when bubbles collapse, even though the delivered energy density was low. However, the distribution of acoustic fields is changed by enlarging of tip diameters. In the experiment with alterable tip diameters of the ultrasonic horn, the reactor geometry was flat-bottomed glass tube with fixed inner diameter of 16 mm, with the rating ultrasonic power density keeping at about 24.4 W/mL [61]. With an increase of tip diameter from 1 to 7 mm, the proportional scale of the surface area of the horn tip to the cross sectional area of the glass inner tube increases from 0.4% to 19.1%. Wide distribution of acoustic fields is given by large emitter surfaces thus this influences the large bulk of enzyme solutions, and is possibly one of the reasons for the unusual behavior of α-amylase under ultrasonic irradiation [62]. Therefore, rational design of scaled-up ultrasonic reactors requires the quantitative prediction of acoustic streaming, power dissipation, mass transfer and cavitational activity in the reactor by theoretical and mapping techniques [34,58].
3) Replacement of a single high-output transducer with an array of transducers that have low output [58]. By using several transducers, the following are favored: i) the formation of more uniform cavitational fields by reducing dead ends of acoustic streaming; ii) intensification of reverberation and superposition effects of ultrasonic energy; iii) reduction in the erosion of transducer tips due to the lower emission power of each transducer.
4) Superposition of ultrasonic field by changing reactor geometries. In a reverberative flow reactor (Table 1), two emission arrays made up of several ultrasonic transducers are mounted on the upside and downside of a metal cuboid reactor, while liquid reactant flows through the inside of the cuboid space [40,54]. Based on the reflection of ultrasonic wave at the inner wall of the reactor chamber and its reverberation in the chamber, the acoustic intensity in this type reactor will be doubled or multiplied, and it will have a more nonuniform cavitation field than single transducer systems. Similar ideas have been adopted in sonochemical polygonal reactors [55,56,60] and innercircular focusing reactors [63].

A properly designed ultrasonic reactor on laboratory- or industrial scale provides a uniform energy field for biomass materials, with a suitable energy level for reactant activation. An advanced sonochemical reactor design is scalable to continuous flow operation and has matching energy efficiency and processing benefit of the laboratory unit.

2.2.2. *Ultrasonic frequency*

Ultrasonic frequency influences the behavior of bubble cavitation through the change in the duration time of acoustic cycle [40], which is short at relatively high frequencies (e.g., 2 μs for 500 kHz) and long for low frequencies (e.g., 50 μs for 20 kHz). High frequency does not favor the occurrence of active cavitation as the time for the growth, radial motion and collapse of bubbles may be insufficient. It also leads to rapid decay of acoustic energy in the liquid medium. However, the influence of ultrasonic frequency on power dissipation level and energy efficiency in cavitation reactors seems to have a number of factors. Some free radical reactions are reported to be accelerated at high ultrasonic frequencies of several hundred kHz [64]. The relatively high frequencies shorten the duration of an ultrasonic cycle, which is accompanied by increase in the number of useful cavitation events in per unit of time [65]. The formed short-life-time radicals may have a higher probability to escape the cavitational bubbles before being quenched [40]. Ashokkumar [29] performed methyl radical recombination experiments in Ar-saturated alcohol solutions at three different frequencies (20, 363 and 1056 kHz). The volume change of bubbles generated during collapse at relative high frequency was much smaller than that in the low-frequency ultrasonic field, which means that much less energy was released at lower collapse temperature. Surprisingly, the determined temperature of cavitational bubbles in water followed the order of 1056 kHz (4730–5930 °C) $>$ 363 kHz (3930–4430 °C) $>$ 20 kHz (3430–3930 °C). This can be explained by the water evaporation that would occur in one acoustic cycle. Longer duration and larger bubble size existing during the radial motion of bubbles in the low-frequency ultrasonic field means that more liquid molecules enter into high-energy bubbles, and then they vaporize, which substantially lowers the actual energy level within the bubbles [29]. Another report demonstrates that the rate of energy release of single bubbles and the cavitational efficiency was higher with ultrasound at higher frequencies by several times [62]. Therefore, there are optimal values for frequency choice in the ultrasonic operation of specific reactions. For the oxidation of ferrous ion, KI and water, the values were 130 kHz, 200–300 kHz and 358 kHz, respectively [33]. However, ultrasonic frequencies higher than 200 kHz should be used with caution as they have adverse effects on energy benefit of the process and on the lifetime of ultrasonic transducers.

Compared with single-frequency ultrasound, ultrasonic reactors with multiple-frequency designs can notably reduce energy loss in the scattering, reflection and absorption of the acoustic waves. Cavitation created with multiple-frequency designs benefits from the low cavitation threshold of low-frequency ultrasound and the high collapse rate of high-frequency ultrasound [66]. Dual- (25 kHz + 40 kHz) and triple-frequency (20 kHz + 30 kHz + 50 kHz) flow cells with bath-type and horn-type devices have been reported to have higher cavitational yields (iodine method) of 7.06×10^{-7} g/J and 5.67×10^{-6} g/J and higher energy efficiencies of 55% and 76%, respectively, much higher than single-frequency device [39,67].

2.2.3. Properties of homogeneous solvents

The physical and chemical properties of solvents greatly influence acoustic propagation and cavitation. The physical properties include the viscosity [68], volatility [69], surface tension of single and multicomponent solvents, while chemical properties include the composition in completely miscible binary/ternary organic mixtures, electrolyte solutions, or surfactant solutions, as well as the chemical activities associated with radical generation.

The formation of cavities in the bulk liquid phase under ultrasonic conditions means the inevitable weakening and breakdown of intermolecular interactions. In the bulk liquid, these interactions are reflected in the physical properties of solvents through changes in viscosity, vapor pressure, boiling point and surface tension. A solvent that has high surface tension and high viscosity probably has an increased energy barrier for the formation of cavities, which is detrimental for cavitation.

In the growth and oscillating stage of cavitational bubbles, the surface tension and volatility affect the size and chemical content of the bubbles. Relative high surface tension of solvents leads to stable bubble sizes that tend not to dissolve in the bulk liquid. High volatility of liquid allows liquid molecules to flow into intensified bubbles and evaporate at negative pressure (rectified diffusion), which reduces the force of bubble collapse. Similar buffering of cavitational intensity occurs when chemical reactions involving volatile reactants or intermediates take place within the bubbles.

The addition of a second homogeneous component in a mixture can have a remarkable influence on the cavitation behavior [29,34,70], because it affects the volatility, surface tension and viscosity of the bulk solution. A second homogeneous component changes both the acoustic streaming and power dissipation in the solution. The addition of 10% (w/v) electrolyte NaCl in water increased the power dissipation by 14.7% and 57.9%, and contributed to an increase in cavitational production by 11.6% and 60.8% at frequencies of 694 and 204 kHz, respectively [34]. The addition of 10% (w/v) NaCl also changes nucleation, growth and collapse of each bubble. The expanding ratio of cavitational bubble size during its growth increased from 6.2 to 8.8 at frequencies of 204 kHz, with the collapse time of bubbles being prolonged from 3.43 to 3.84 μs [34]. The introduction of a non-volatile salt changes the volatility and partitioning behavior of solvents. For the sonochemical degradation of volatile chlorobenzene and non-volatile phenol, the introduction of 4% (w/v) NaCl enriched the organics at the interface between the bubble and bulk liquid, with the partition coefficient of chlorobenzene and phenol increased from 29.2 to 48.0 and from 3.3 to 11.7, respectively [69]. The molar concentrations of chlorobenzene and phenol vapors entrapped in the bubbles increased from 77.1 to 79.8 ppm and from 0.96–1.90 to 3.55–6.94 ppm, respectively [69]. The most active zone for sonochemical reactions is normally regarded to be the inner of cavitational bubbles and the thin liquid shell that surrounds the bubbles. As a result, the degradation efficiency of phenols increased to three-fold.

The addition of surfactants reduces the dissolution and coalescence of bubbles in multibubble systems [29,70]. The sizes of cavitation bubbles in 1 mM sodium dodecyl sulfate solution were significantly reduced compared with those in water [29].

The physical properties of the liquid medium influence the delivery and dissipation of acoustic power to the solution. Detrimental attenuation of acoustic energy occurs in viscous mediums such as in plant oil [40], which decreases the process benefit of ultrasonic operation.

Physical properties of homogeneous solvents have many effects on ultrasonic cavitation. Low viscosity, relative low volatility and relative high surface tension of liquid solvents are regarded as favorable for achieving efficient cavitation. Frequently, water is used as solvent in most ultrasonic operations as it has a higher cavitational intensity than common organic liquids [28].

2.2.4. Heterogeneous properties of ultrasound-assisted reactions

Active cavitation in ultrasound-assisted reactions is generally preferred to occur at the heterogeneous sites in liquids. These heterogeneous sites can be impurities, free gas microbubbles in the liquid, new nuclei due to collapse and fragmentation of bubbles in the previous acoustic cycle, and crevices at the heterogeneous interface such as reactor wall or non-volatile additives [28,71]. In the acoustic emission spectrum of pure water without any impurities or gas microbubbles, only one peak, corresponding to the fundamental frequency of the ultrasound is seen, which means that no cavitation occurs for pure water [32]. On the contrary, for water containing a certain concentration of gas nuclei (microbubbles in the reactor crevices), strong subharmonic, ultraharmonic and harmonic emissions appeared in the acoustic emission spectrum.

The existence of dissolved gas affects the behavior of bubbles and thus the cavitation intensity in sonochemical reactors. For •OH radical reactions, the order of the rate constant of •OH radical formation (μmol/(L min) at an ultrasound frequency of 513 kHz under different gas atmospheres was Kr (3.17) > Ar (2.90) > O_2 (2.68) > He (0.58) [64]. Gaseous He had the lowest rate constant that could be attributed to its high thermal conductivity compared with other gases [72]. When the dissolved gas has high thermal conductivity, more energy in the cavitational bubbles is transferred to the surrounding solution, while concurrently less energy is obtained as the bubble collapse. The solubility of gas in water ($\mathrm{Kr} \gg \mathrm{Ar} \approx \mathrm{O_2} \gg \mathrm{He}$) is also a factor in the results [72]. Low gas solubility in water does not provide enough nuclei for ultrasonic cavitation, while high gas solubility in water might weaken the cavitation effects as the formed cavitational bubbles may redissolve before their collapse [40].

For specific biological or chemical processing such as emulsification [73], microbial cell disruption [74], enzyme activation [61] and radical formation [64], appropriate amounts of dissolved gas provide nuclei for the production of cavitational bubbles, and favor ultrasonic cavitation. For emulsification, no clear effect of gas content is observed on the size of emulsion droplets for a constant energy density [73]. However, for processing of microbial cells, the rate constant of α-amylase deactivation was relatively lower in pre-degassed water [61]. Bubbling with CO_2 or (CO_2 + H_2) during sonication enhanced the release of polysaccharides from *Porphyridium sp.* cells [75].

The presence of foreign additives, including volatile and non-volatile substances, not only lowers the very high-energy threshold for cavitation in the pure solvents, but also seems to induce chemical effects in acoustic cavitation through the promotion or inhibition of radical formation. Katekhaye and Gogate [71] determined the effect of some additives on cavitational yield with a horn-type reactor (20 kHz, power dissipation of 0.098 W/mL by calorimetric measurement) using the iodine method. They used solid particles (CuO, TiO_2), salts (NaCl, $NaNO_2$) and radical promoters (H_2O_2, $FeSO_4$, metal iron, CCl_4 and *tert*-butanol). All additives showed a positive influence on cavitational yield with the improvement being 40–240%, while the addition of radical promoters such as *tert*-butanol, CCl_4 and $FeSO_4$ showed the best results. With proper combination of several additives (such as $TiO_2 + H_2O_2$), synergistic effects can be seen with much higher cavitational yields than those for using individual additives. However, for some additives such as TiO_2, H_2O_2 and *tert*-butanol, high concentrations can be detrimental to cavitational yield. This might be interpreted as the enhancement of the premature rupture of cavitational bubbles or the recombination of free radicals at high concentrations of these additives, while the detailed mechanism needs further investigation.

As highlighted in Section 2.1, the level and efficiency for cavitation energy depends not only on the transformation of acoustic energy to cavitation energy, but also is related to the attenuation and energy absorption of the acoustic wave propagating in the system. For instance, high density of bubble clouds may be generated at the zones with high ultrasound intensities, such as the region near the tip of the transducer. The bubble clouds could absorb or scatter acoustic waves, and result in inefficient energy delivery within the reactor [61]. Cavitation energy is also absorbed by solvents and heterogeneous system. The amplitude of pressure pulse measured at 1 mm from the bubble center was only one-in-one-thousands of its actual value [35]. Similar attenuation of acoustic energy and cavitation energy might be seen in slurries or suspensions such as insoluble biomass particles, humin products and nano-scale catalysts. Therefore, additional care must be taken for treating these type systems [58]. There are two conflicting aspects regarding the presence of impurities: (i) promotion of cavitation by impurities and (ii) acoustic attenuation by the scattering of acoustic energy when the acoustic wave bypasses the impurities. As a result, the existing and concentration of heterogeneous impurities should have a reasonable value, which depends on the size and properties of impurities. However, qualitative and quantitative information about acoustic attenuation in heterogeneous systems is still lacking and need to be studied in future.

2.2.5. Other parameters

For acoustic cavitation, other factors related to the sonochemical environment include the temperature of medium, the static pressure of the fluid [73,76] and the level of power dissipation in the solution. The details can be seen in the following sections and other available literature [28,33,40]. However, the selection and optimization of these parameters considers not only acoustic cavitation, but also the requirements for raw materials and processing.

In much of the content presented, power ratings of acoustic levels are given to describe the systems used in the studies. The rating values might be viewed as a specification of sonochemical reactors. However, it should be noted that these power ratings are not practical power levels, since there are losses in the energy transformation. The actual power level in the reactant solutions is normally 10–40% of the rated values [71,77–80]. Actual power levels can be determined with calorimetric measurements. For these cases, the determined values are specifically noted as “by calorimetric method” in this review. The power levels for ultrasonic processing are largely related to the scale or volume of processing. Therefore, the values of mean power density (the ratio of acoustic power to the volume of liquid reactant) are preferentially considered and listed in this review as a substitute for raw power values. The volume of liquid reactant is defined as the actual volume of liquid reactant in the reaction vessel for probe-type reactors, while it is the volume of the water bath in the reactor for sonication, as the surrounding liquid in the tank is the first receptor of ultrasonic irradiation.

Factors that influence the energy level of cavitation and the energy released during cavitation to the reactants are related to the size change in bubble radial motion, the lifetime of bubbles, the collapse time of bubbles and the chemical compositions in the bubble. While higher acoustic intensity and lower threshold for cavitation may result in more population of active bubbles. For different intensification mechanism, the influence of factors may be different. For shockwaves and micro-convection induced intensification, the intensity of shockwaves and micro-convection is related to the intensity and rate of energy release during bubble collapse, and thus is depended on the violence of the extending and shrinking of bubble size, the evaporation of bubble contents, the adiabatic property of liquid and the time for bubbles collapse. For chemical radical induced intensification, it is important to ensure sufficient volatile substrates trapping in the bubbles or staying at the thin liquid shell that was very close to the bubbles. It is also important to shorten the time for bubbles collapse and enhance the escape of radicals from the bubbles. For non-volatile reactants, the absorption of cavitation energy by viscous liquid or heterogeneous system should be also carefully considered. Therefore, the optimization of ultrasonic parameters should consider the specific reactions and the real intensification mechanism.

3. Ultrasonic pretreatment and reactions of lignocellulosic biomass

Lignocellulosic biomass is one of the most abundant bio-resources in the world, with an annual world growth of 170–200 billion tons [2]. The structure of lignocelluloses is complex and recalcitrant, with cellulose (40–50%), hemicellulose (10–20%) and lignin (20–30%) as the three most abundant components. The primary requirements for the thermochemical and biochemical conversion of lignocelluloses are:

1) Pre-fractionation of raw lignocelluloses before reactions. Lignin and hemicellulose should be firstly removed from cellulosic materials, as the raw lignocellulosic complex hinders the access of enzyme molecules and chemical catalysts. The critical issue is how to improve the efficiency and economics in the destruction of lignocellulosic structure and the separation of the connected components at mild condition. The reduction in the reaction severity also means less production of by-products (such as phenols, furfurals and organic acids) as well as less alteration of the glucosidic structure of cellulose.
2) Opening of the crystalline structure of raw cellulose at mild conditions. Compared with lignin and hemicellulose, the regularity in the chemical and crystalline structure is the most prominent characteristic of cellulose. Hundreds to tens of thousands of D-glucopyranoses constitute the molecular chains through $\beta(1 \rightarrow 4)$ glucosidic linkages with unified orientation, while most chains participate in the recalcitrant crystalline region of raw cellulose by intra and intermolecular hydrogen bonds. This makes cellulose strongly hydrophobic, and difficult to be dissolved or degraded into common solvents. In the degradation dynamics of cellulose, the lowering and destruction of its crystalline structure requires extremely high activation energy. For example, the energy required for removing a glucan from crystalline cellulose in room temperature water is calculated to be about 8.1 kJ/mol [81], which influences the saccharification efficiency and hydrolysis selectivity.

3) Intensification of mass transfer in heterogeneous reactions containing solid lignocelluloses. The surface of lignocelluloses needs to be increased through pretreatment for better contact with solvents for reactions.

To meet these requirements, pretreatment and reactions of lignocelluloses should be performed in surroundings that provide sufficient reaction intensity such as high-energy input to destroy lignocelluloses, but remain at relatively mild conditions and low energy intensities such as room temperature, short treatment time and neutral pH values of the solution for the purposes of atom efficiency. It seems to be contradictory, however, ultrasonic pretreatment can address these requirements, because ultrasonic treatment features high local strength with its intense cavitation and overall moderate effects. However, much progress has been reported on ultrasound-assisted lignocellulose fractionation, component solvation, thermochemical/biochemical conversion especially for saccharification and fermentation. Progress on these topics will be reviewed in this section.

3.1. Destruction and fractionation of lignocellulose

Ultrasonic energy applied as pretreatment has special mechanistic effects on the structural integrity of lignocelluloses (Fig. 1). Ultrasonic treatment can destroy wax layers and silica bodies deposited onto the surface of lignocellulosic structures and can aid in their removal [82,83]. The size of biomass particles can be reduced when subjected to high power ultrasound [84–87]. Cellulosic materials are crushed to particles or crystalline grains having micro- or even nano-size ranges after being subjected to a mean rating power of 3.0–10.0 W/mL [88,89]. Sonotreatment of chemically-separated cellulose in water at 6.67 W/mL for 30 min gave nano-sized fibers with diameters of lower than 20 nm [88,90,91]. Pinjari and Pandit [86] reported their work on the milling of natural cellulose to nanofibrils using hydrodynamic and ultrasonic cavitation in succession. Sonication at 3.0 W/mL for 110 min reduced the size of the cellulose particles from 1360 nm after hydrodynamic cavitation to 301 nm. High-intensity ultrasound also increases the surface area of solid biomass by cavitation erosion [56,92]. As a result, size reduction and erosion of lignocellulose particles benefits the extraction of chemicals from biomass and the saccharification of cellulosic materials [85,87].

Application of ultrasonic energy aids in the dissolution or solvation of cellulose, hemicellulose and lignin in organic solvents and ionic liquids. Ultrasound accelerates the dissolution of cellulose. With ultrasound pre-irradiation for 20 min and subsequent heating without ultrasound for another 60 min at 110 °C, the dissolution of cellulose in ionic liquid 1-butyl-3-methylimidazolium chloride ($[C_4mim]Cl$) was complete, as compared with 190 min for conventional heating only [93]. Dissolution or extraction of lignin from bagasse or bamboo powders in 1,4-butanediol/water mixtures [94] or 95% ethanol [95] is more effective with ultrasonic energy than conventional agitation.

Sonication has been used to achieve favorable results in the fractionation of raw lignocelluloses [14,96] and extraction of compounds in raw biomasses [16] such as polysaccharides, phenolic

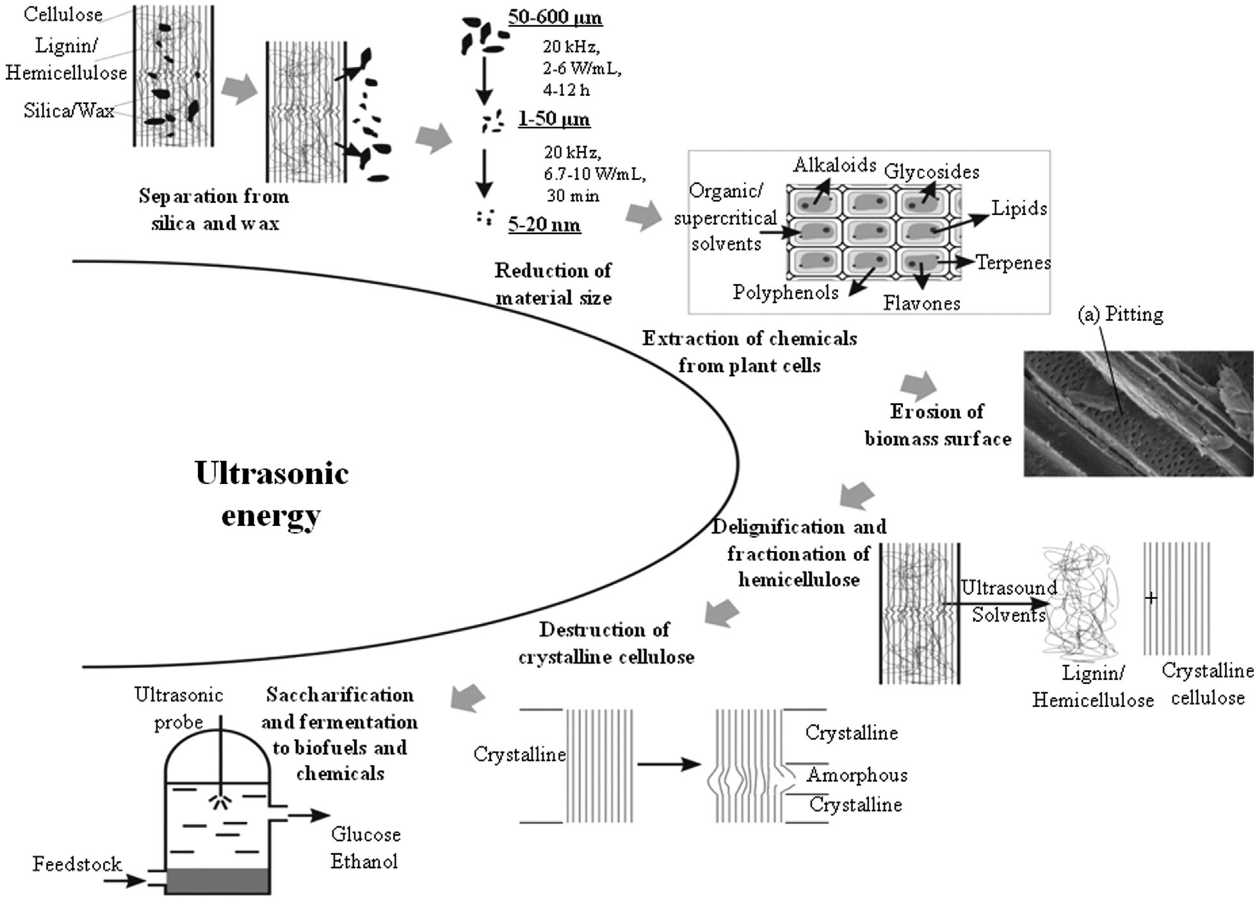

Fig. 1. Mechanistic effects of ultrasonic energy (US) in lignocellulose pretreatment and conversion [14,16,56,82–84,86,88,100,102] (a: Reprinted with permission from Ref. [56], Copyright ©2011 Springer.)

Table 2
Ultrasound-assisted pretreatment and reactions of starch, cellulose and lignocellulose.

Materials	Ultrasonic process and parameters[a]	Consequent process and parameters	Key findings	Ref.
Sugary-2 maize	**Pretreatment:** Probe, 20 kHz, 4.8–8.3 W/mL, 5–40 s, Concentration of 3 g/32 mL	**Starch hydrolysis:** 32 °C 3 h, *Stargen™ 001*	Starch conversion improved from 13.7% to 39.2–56.8%.	[116]
Corn meal	**Pretreatment:** Sonicator, 40 kHz, about 1.58 W/mL, 60/80 °C, 3–10 min, Sample concentration of 25 wt.%	**Starch liquefaction:** 85 °C, 1 h, Solid/water weight ratio of 1/3 *Termamyl SC*, pH 6.0 **Simultaneous hydrolysis and fermentation (SSF):** 30 °C, 32 h, *Saccharomyces cerevisiae var. ellipsoideus*, *SAN Extra L*, pH 5.0	1) Glucose yield increased from 29.6 wt.% to 31.6 wt.% after liquefaction. 2) Bio-ethanol concentration increased from 0.45 g/g starch to 0.50 g/g after fermentation.	[115]
Microcrystalline cellulose	**Pretreatment:** Probe, 20 kHz, 1.5 W/mL, 80 °C, 3–9 h, Solid/liquid ratio of 5 g/200 mL	**Microwave-assisted hydrolysis over solid catalyst:** Microwave power of 350 W, 150 °C, 1 h, Solid/water ratio of 0.5 g/ 5 mL, Sulfonated carbon	1) Size of cellulose particles reduced from 38 μm to about 0.3 μm. 2) Glucose yield promoted from 3.8–4.9% to 11–21%, comparable with ball milling or ionic liquid pretreatment. 3) High selectivity of 64–71% for hydrolysis, without by-product such as furfurals. 4) Much shorter time of <1 h for cellulose hydrolysis.	[87]
Triticale meal	**Pretreatment:** Bath, 40 kHz, about 0.05 W/mL, 40–60 °C, 5 min, Sample concentration of 25 wt.%	**Starch liquefaction:** 60 °C, 65 min **SSF:** 30 °C, 72 h, *Saccharomyces cerevisiae* yeast	1) Glucose and maltose yields increased by 12.3–15.7% and 46.7–52.6% after liquefaction. 2) SSF time reduced from 72 h to <48 h, with bio-ethanol content increased by 8.2–10.9%.	[123]
Cassava chip	**Pretreatment:** Probe, 20 kHz, 8.5 W/mL, 10–30 s, Slurries with 5% total solids.	**Simultaneous liquefaction-SSF:** 32 °C, 72 h, *Stargen™ 001* + *Saccharomyces erevisiae (ATCC 24859)*	1) Ethanol yield increased from 31.4% to 43.1% after sonication at 8.5 W/mL for 30 s. 2) Fermentation time reduced from 72 h to 36 h.	[118]
Oil palm fruit bunch	**Pretreatment:** Probe, 20 kHz, about 2–3 W/mL, 25 °C, 15–45 min, Solid/liquid ratio of 50 g/500 mL, 2% H_2SO_4	**Hemicellulose hydrolysis:** 100–140 °C, <200 min, Solid/liquid ratio of 1:25, 2% H_2SO_4	Xylose yield increased from 22% to 52%.	[83]
Switchgrass	**Pretreatment:** Hexagonal reactor, 20 kHz, 50 °C, 5 h, Solid/0.1 M CH_3COONa buffer ratio of 10 g/250 mL	**Cellulose enzymatic hydrolysis:** 50 °C, 5 h, Solid/0.1 M CH_3COONa buffer of 10 g/250 mL *Accellerase 1500®*	Reducing sugar yield increased from 8 wt.% of control group to 9.75 wt.%.	[56]
Rice hull	**Pretreatment:** Bath, 40 kHz, about 0.025 W/mL, 25 °C, 30 min, Solid loading of 15% (*w*/*v*)	**Cellulose fungal pretreatment:** 28 °C, 18 days, Solid/liquid ratio of 35/65, *P. ostreatus* **Enzyme hydrolysis:** 45 °C, 48 h, Enzyme, pH 4.8	Net yields of total soluble sugar and glucose were 31.8% and 32.2% compared with control group values of 6.9% and 7.6% (sole fungal pretreatment for 18 d).	[121]
Areca nut husk (*Areca catechu*), bon bogori (*Ziziphus rugosa*), moj (*Albizia lucida*)	**Lime pretreatment:** Probe, 30 kHz, 100 W (36 W, calorimetry), 35 °C, 1–3 h, Biomass loading of 3.2–9.0 wt.%, Lime, 0.5 wt.% of biomass.	**SSF:** 35 °C, 72 h, Biomass loading of 10 wt.%, Cellulase (*Accellerase 1500)* + *Saccharomyces cerevisiae*, pH 4.5	1) Delignification ratios increased from 50–54% to 64–68%, with time reduced from >8 weeks to 1.5–3 h. 2) Ethanol yields, 73.7% for areca nut husk, 83.1% for bon bogori and 85.2% for moj.	[77]
Kenaf core fiber	**Ionic liquid pretreatment:** Probe, 24 kHz, about 8 W/mL, 25 °C, 15–30 min, 1-Ethyl-3-methylimidazolium acetate (5 g /0.25 g material)	**Cellulose enzymatic hydrolysis:** 50 °C, 48 h, Cellulase (from *Trichoderma viride*), pH 5.0	Saccharification ratio increased from 47% to 86%.	[125]
Microcrystalline cellulose	**Ionic liquid pretreatment:** Bath, 45 kHz, about 0.01 W/mL, 60 °C, 30 min, Alkylimidazolium dimethylphosphate ionic liquid ([Mmim][DMP])	**Cellulose enzymatic hydrolysis:** 50 °C, 24 h, [Mmim][DMP] concentration of 20%, Cellulase (from *Trichoderma reessei*), pH 4.8	Cellulose conversion improved from 75.6% to 95.5% with ultrasound.	[126]
Microcrystalline cellulose, bamboo	**Ionic liquid pretreatment:** Probe, 24 kHz, 1.7 W/mL, 25 °C, 60 min, Choline acetate	**Material regeneration in water** **Enzymatic hydrolysis of recovered material:** 50 °C, 48 h, Cellulase (from *Trichoderma viride*), pH 5.0	Saccharification ratio improved to 92.4% from 55.0% with ionic liquid pretreatment only.	[127]
Kenaf core fiber	**Sonocatalytic-Fenton pretreatment:** Probe, 24 kHz, 0.7 W/mL, 25 °C, 3–6 h,	**Cellulose enzymatic hydrolysis:** 50 °C, 48 h, Kenaf powder loading of 2% (*w*/*v*), Cellulase (from *Trichoderma viride*), pH 4.0	Saccharification ratio promoted to 22.4–24.3% of sonocatalytic-Fenton treatment from 9.8 to 11.0% of control group, 13.0 to 14.0% of sonocatalytic	[124]

Table 2 (*continued*)

Materials	Ultrasonic process and parameters[a]	Consequent process and parameters	Key findings	Ref.
	$\mathrm{TiO_2}$ amount of 2 g/L, Fenton reagent ($\mathrm{H_2O_2}$, 100 mM, $\mathrm{FeSO_4}$, 1 mM)		treatment and 14.9 to 15.3% of Fenton treatment.	
Carboxymethyl Cellulose (CMC)	**Pretreatment:** Probe, 20 kHz, 0.72–1.35 W/mL, 50 °C, 20–60 min pH 4.8	**Enzyme hydrolysis:** 50 °C, CMC concentration of 10 g/L, Mixed cellulases from *Trichoderma viride* and *Aspergillus niger*, pH 4.8	1) Maximum reaction rate over mixed cellulase (70 wt.% *Trichoderma viride* + 30 wt.% *Aspergillus niger*.) increases from 6.94×10^{-7} to 9.86×10^{-7} mol/(L s) with ultrasound. 2) Maximum reaction rate over *Aspergillus niger* did not increase with ultrasound.	[120]
Waxy rice starch	**Starch hydrolysis:** Homogenizer, 20 kHz, up to 0.12 W/mL, 60 °C, 24 h, Concentration of 1%	–	Number-average molecular weight increased from 1.3×10^{-5} to 0.2×10^{-5}.	[117]
Maize starch	**Starch acid hydrolysis:** Probe, 25 kHz, about 4 W/mL, 90–100 °C, 0.5–2 h, $\mathrm{H_2SO_4}$ (3–5 wt.%)	–	Glucose yield of 32–97 wt.%, 22–100% higher than control group.	[128]
Glucose	**Glucose fermentation:** Bath, continuous, 40 kHz, about 0.027 W/mL, 20 or 30 °C, 4–20 h, Glucose (20–40 wt.%), *Saccharomyces cerevisiae*	–	Reaction rate constant increased by 1.3–1.5 times compared with stirring.	[129]
Sugarcane bagasse (SCB)	**Alkaline pretreatment:** Probe, 24 kHz, 2.0 W/mL, 50 °C, 20 min, Solid/liquid ratio of 1 g/20 mL, 2% NaOH **Acid hydrolysis:** Pulse, cycle control (50% cycle), 50 °C, 30–45 min, Solid/liquid ratio of 1 g/20 mL, 2% $\mathrm{H_2SO_4}$	**Hydrolyzate fermentation:** 30 °C, 60 h, *Saccharomyces cerevisiae*, initial pH of 5.0.	1) Cellulose and hemicellulose recoveries of 99% and 79%, lignin removal of 75% after alkaline pretreatment. 2) Maximum ethanol yield of 0.47 g/g glucose, 0.17 g/g of pretreated SCB.	[100]
Newspaper, office paper, pulp	**Cellulose enzymatic hydrolysis:** Probe, 0.8–6.4 L reactor, 20 kHz, 0.005–0.075 W/mL (calorimetry), 45 °C, 48 h, Substrate concentration of 7.5–25 g/L, Cellulase (from *Trichoderma viride*), pH 4.8	–	Apparent rate constants increase from 11–13 to 16–24 $\mathrm{h^{-1}}$ for 0.8 L reactor, from 10–14 to 15–19 $\mathrm{h^{-1}}$ for 3.2 L reactor, from 8.5–14 to 14–18 $\mathrm{h^{-1}}$ for 6.4 L reactor.	[78]
Sugarcane bagasse	**Alkaline pretreatment:** Probe, continuous, 24 kHz, up to 2.0 W/mL, 40 °C, 30 min, Solid/liquid ratio of 1 g/20 mL, 2% NaOH **Enzymatic hydrolysis:** Probe, interval, 24 kHz, up to 2.0 W/mL, 40 °C, 6 h (ultrasonic for 3 h), Solid/liquid ratio of 1 g/15 mL, *Cellulomonas flavigena (MTCC 7450)*, pH 6.0	**Hydrolyzate fermentation:** 30 °C 48 h, *Zymomonas mobilis (MTCC 89)*, pH 5.7.	1) After alkaline pretreatment, 80.8% of hemicellulose and 90.6% of lignin removed. 2) Glucose yield after enzymatic hydrolysis increased from 34.5 wt.% of control to 57.6 wt.%. 3) Ethanol yield increased from 43.7 g/g glucose to 46.6 g/g.	[102]
Avicel cellulose, sugarcane bagasse	**Pretreatment:** Bath, 45 kHz, about 0.01 W/mL, 80 °C, 72 h, Substrate concentration of 3% (*w*/*v*), *N*-methylmorpholine-*N*-oxide (NMMO) **Cellulose enzymatic hydrolysis:** Bath, 45 kHz, about 0.01 W/mL, 50 °C, 12–48 h, Substrate concentration of 0.6% (*w*/*v*), *β*-glucosidase (from *Aspergillus niger*), pH 4.8	**Hydrolyzate fermentation to biodiesel:** NMMO, 20% (*w*/*v*) 30 h, *R. opacus ACCC41043*	Conversions of avicel cellulose and sugarcane bagasse at hydrolysis time of 12 h, 1) Untreated samples, 28% and 16%. 2) NMMO-treated samples, 72% and 72%. 3) NMMO-ultrasound-treated samples, 96% and 91%.	[132]

[a] Power levels additionally marked with word “calorimetry” is actually determined using calorimetry method, while others without labels are the power rating of the device.

compounds, seed oils and other valuable products. Shockwaves and high-speed microjets of ultrasonic cavitation impact, loose and destroy the chemical linkages in the lignocellulosic structure. Substrates such as hemicellulose and lignin were destructively partitioned from cellulose and entrapped in the interbundles of cellulose microfibril. The violent small-scale flows at the immediate vicinity of microfibers caused by cavitation then sufficiently dislodge out these substrates [97]. The most studied reagents in the removal of hemicellulose and lignin under sonication are 0.5–2% NaOH or KOH [98–102], alcohol [98,99], lime [77], H_2O_2 and surfactants [103] such as Tween-40. Sun et al. [98,99] found that lignin and hemicellulose were separated more completely from wheat straw by ultrasound-assisted extraction with 0.5 M NaOH in 60% aqueous methanol at 60 °C. Sonication released 77.3% lignin and 40.8% hemicellulose within 20 min as compared with 61.0% lignin and 32.2% hemicellulose in the absence of ultrasound [99]. Ultrasonic pretreatment assists in destroying lignocellulosic structures and lowering the molecular weight of lignin and hemicellulose. However, the primary chemical structural features of lignin and hemicellulose remain. Velmurugan and Muthukumar [100] and García et al. [101] reported similar results for sugarcane bagasse (SCB) and olive tree biomass. After sono-assisted alkaline pretreatment (SAP), 21.1% hemicellulose and 75.4% lignin in bagasse were extracted and degraded in the liquid phase, but only 0.8% cellulose was removed [100]. Due to the removal of hemicellulose and lignin in the amorphous regions, a high crystallinity of cellulose was obtained, accompanied with improved thermal stability of the materials [88,100]. Additionally, considering the heterogeneity of the chemical structure in amorphous hemicellulose and lignin, ultrasound was also used in the fractionation of hemicellulose and lignin into various compositions that discriminated in molecular weight and chemical structures, by combining ultrasound with different organic solvents or NaOH aqueous solutions with different concentrations [104,105].

For cellulose, high-intensity ultrasonic cavitation destroys the intermolecular hydrogen bonding of crystalline cellulose. The crystallinity and the polymerization degree of cellulose are substantially decreased after sonication, accompanied with an increase in hydrophilicity and reactivity [86,106,107], which improves the accessibility of substrates to enzymes or chemical catalysts. For cellulose (1.3 μm) that is derived from hydrodynamic treatment, the crystallinity of cellulose decreases from 60.7% to 37.8% after subsequent sonication [86]. This made it easier for the recovered cellulose nanofibrils to be degraded. Wang et al. [106] showed similar trends for microcrystalline cellulose. The cellulose was activated after sonication, with remarkable decrease of crystallinity and increase of water retention value, which is an index for the accessibility of cellulose for water molecules [106]. Similar study with cellulose microfibrils from *Glaucocystis* (a kind of *Cyanophyta* algae) further showed the transformation of cellulose crystalline state under sonication [108]. After sonication of 0.1 wt.% microfibril suspension at 4 °C and rating power of 1.67 W/mL for 3 h, the ratio of β-cellulose to α-phase changed from 10/90 to 64/36.

On the other hand, the influence of ultrasonic treatment on its chemical and crystalline structure might depend on the treated materials and ultrasonic power. Sonication of chemically-purified fibers (10–100 nm) does not seem to have significant effects on the crystalline regions of the cellulose nanofibers [88]. High power input tears the high-strength hydrogen-bonding network in cellulose, but too much power input may result in unwanted degradation of amorphous regions, and result in the enhancement of the crystallinity of cellulose [88].

Unlike cellulose and hemicellulose, sonication may have a detrimental effect on lignin structure and its activity [109–111]. The structure of lignin after sonication seems to be more stable and this is due to compaction of the bonding networks within lignin in aqueous solutions [109,112]. Sonication for 30 min resulted in remarkably condensed structures of alkali lignin, for which the number-average and mass-average molecular weight increased from 1179 and 10,250 to 10,649 and 42,325, respectively [110]. The content of hydroxyl groups of lignin increased from 3.86 to 6.67 mmol/g after sonication for 20 min, but decreased to 5.16 mmol/g after sonication for 30 min.

In industrial-scale production of biofuels from lignocellulosic materials, pelleting is a necessary step to adapt the random stacked bundles or powders into uniform and compact shapes that have high-energy density per unit volume. Pelleting allows transport of raw materials and improves the energy efficiency in conversion processes. Zhang et al. [113] found that with the help of ultrasonic energy, pelleting of lignocellulosic materials could become more efficient. Ultrasonic vibration-assisted pelleting at 20 kHz produced uniform switchgrass granules of 0.2–1.2 mm that had higher density and stability than those without ultrasound treatment. High sugar yields of 86.4% were obtained for subsequent 2% sulfuric acid pretreatment and enzymatic hydrolysis (*Accellerase 1500*, 50 °C, 72 h) of the pellets, which was higher than the yields without pelleting (69.9%) or pelleting without ultrasound (70.3%).

The introduction of high-intensity ultrasound benefits the solvation, delignification, fractionation and extraction of compounds in raw lignocellulosic biomass by strong physical effects such as shockwave, microjets and micro-convection. Ultrasound helps in increasing surface area of solid biomass through the reduction of biomass size and cavitation erosion, although the change of solid size may result in new problems such as acoustic attenuation. The crystalline structure of cellulose will be changed or destroyed by high-intensity ultrasonic cavitation, which increases the hydrophilicity, accessibility and reactivity of sonicated cellulose. However, for lignin material, sonication is probably better used for producing novel polymer materials with enriched C5 condensed phenolic structures [114].

3.2. Conversion of lignocellulose to saccharides and bio-ethanol

Sonication improves hydrolysis of lignocellulosic materials into sugars and their subsequent fermentation to bio-ethanol. Detailed examples are listed in Table 2.

Degradation of starch from different sources such as waxy rice, corn meal, maize, potato and cassava chip (Table 2) is promoted by ultrasonic pretreatment [17,115–119]. By sonication pretreatment for 5 min, glucose concentration after liquefaction of corn meal and bio-ethanol concentration after simultaneous saccharification and fermentation (SSF) increased by 6.8% and 11.2%, respectively [115]. For sugary maize, sonication at 4.8–8.3 W/mL for 5–40 s greatly enhances the swelling of the samples, and thus improves enzymatic hydrolysis toward fermentable sugars by about two–three-fold [116]. The increase of theoretical starch conversion was highest with high ultrasonic power density (8.3 W/mL), but the energy efficiency, which was defined as the promotion of starch conversion per unit power input, decreased. One concern in the sonication of starch is the limit for high total solid concentrations as a result of its gelatinization [116].

Application of ultrasonic energy greatly increases the hydrolysis yield of hemicellulose to xylose [83]. Compared with 22% for the control groups, palm empty fruit bunch fibers produced xylose yields of 52% for hydrolysis reactions at 100 °C with 2% sulfuric acid as catalyst after sonication at about 3 W/mL for 45 min, while its cellulose part was less degraded [83]. The increase of ultrasonic power, as well as the increase of the duration during ultrasonic treatment, greatly influences hydrolysis efficiency, while longer durations at relative low power density of about 0.5 W/mL did not have significant effects. This implies that acoustic power should be properly selected to ensure sufficient energy intensity for the activation of the desired reactions.

Ultrasonic pretreatment is used in thermochemical and biochemical reactions of cellulosic materials to produce glucose and bio-ethanol [102,120–123]. Sonication of microcrystalline cellulose at rating power of 1.5 W/mL for 3–9 h generates a milk-like colloidal suspension that is stable for over three months with high cellulose concentration up to 50 g/L [87]. The cellulose after sonication showed much better performance in the microwave-assisted hydrolysis at 150 °C over sulfonated carbon solid catalyst, with glucose yield of 11–15% and no detection of secondary degradation from sugars. Ultrasound provides very high energy of approximately 10–100 kJ/mol, which is sufficient for destroying the microfibril structure of microcrystalline cellulose. However, according to the results from Fourier transform infrared spectroscopy, thermal gravimetric analysis and intrinsic viscosity measurement, the chemical structure, thermal stability and the degree of polymerization of recovered cellulose show no obvious alteration after sonication. The degree of crystallinity decreased from 78.4% to 66.3%, but mean particle size of cellulose was dramatically reduced from 38 μm to less than 0.4 μm after sonication [87]. Therefore, the reduction of particle sizes, the dispersion of submicron particles, as well as the possibly increase of hydrophilicity of the sonicated cellulose that results from partial disruption of hydrogen-bonding networks, can be regarded as the main reason for stable storage of milk-like colloidal suspensions and promotion of cellulose hydrolysis. Interestingly, compared to the recovered and dried materials, the colloidal suspension without further treatment could reach a higher glucose yield of 21%. This may be attributed to the result of possibly agglomeration and the loss of the hydrophilicity of particles during the recovery of sonicated cellulose from the suspension, which made the accessibility of cellulose particles to solvent and catalysis harder. For the sonication of raw switchgrass in a hexagonal ultrasonic reactor before its enzymatic hydrolysis, similar benefits of ultrasonic pretreatment were also reported [56].

Integration of ultrasonic energy with other methods such as alkali treatment (2% NaOH [102], lime [77]), TiO_2–Fenton pretreatment [124], biological digestion or ionic liquid dissolution seems to be effective for the pretreatment of lignocelluloses. Sonication in lime pretreatment of three types of biomass, areca nut husk, bon bogori and moj obtained significantly high delignification ratios within much shorter times (3 h *vs.* 8 weeks) than the conventional process, which was favorable for the SSF process over cellulase (*Accellerase 1500*) and *Saccharomyces cerevisiae* [77]. Sonocatalysis in the Fenton pretreatment (Fe^{2+}/H_2O_2) accelerated and promoted the degradation of lignin part, which increased the yield of subsequent saccharification [124]. Combination of ultrasonic energy with fungal treatment efficiently disrupted the lignin structure, and enhanced the enzymatic hydrolysis of rice hulls [121]. Yields of total soluble sugar and glucose after the successive sonication (30 min) and fungal (*Pleurotus ostreatus*, 18 d) pretreatment were 3.6 and 3.2 times higher than that of sole fungal pretreatment (18 d), respectively.

Combination of ultrasonic energy with ionic liquids has attracted much interest for the conversion of cellulosic materials [125–127]. Ionic liquids and organic reagents such as *N*-methylmorpholine-*N*-oxide (NMMO) are good solvents for cellulosic materials, and could destroy substantial portions of the microcrystalline structure of cellulose. As a result, the advantage of combined treatment of sonication with ionic liquids is the reduction of excess high ultrasonic energy or heating energy to destroy the recalcitrant crystallinity of cellulose. This improves the energy economics of ultrasonic operation, and possibly reduces the formation of by-products such as furfural.

The solvation of cellulose by ionic liquids depends on the properties of ionic liquids, while it is accelerated and promoted under ultrasonic irradiation. The sonication in ionic liquids, 1-butyl-3-methylimidazolium chloride (BmimCl), 1-allyl-3-methylimidazolium chloride (AmimCl), 1-ethyl-3-methylimidazolium chloride (EmimCl), 1-ethyl-3-methylimidazolium diethyl phosphate (EmimDep) and 1-ethyl-3-methylimidazolium acetate (EmimOAc) at 25 °C for 15–120 min achieved higher enzymatic saccharification yields of kenaf cellulose than conventional thermal pretreatment in ionic liquids by 80–470% [125]. The crystallinity index of cellulose was effectively decreased after sonication, while the chemical structure of cellulose was only slightly affected. However, sonication of cellulose in alkylimidazolium dimethylphosphate ionic liquids such as 1-methyl-3-methylimidazolium dimethylphosphate ([Mmim][DMP]) at 60 °C resulted in partial depolymerization of cellulose [126]. Although the high viscosity of ionic liquids may result in the attenuation of acoustic intensity in the reaction systems, it is also recommended to perform ultrasound-assisted ionic liquid pretreatment of cellulose at low ultrasonic power and ambient temperature, as the main purpose of sonication should be the enhancement of the dispersion and solvation of cellulose in ionic liquids, rather than the promotion of erosion of crystalline structure by high-intensity cavitation.

Ultrasonic energy can have a direct influence on hydrolysis and fermentation reactions. Under ultrasonic conditions, starch becomes depolymerized to glucose at 100 °C, with 5 wt.% sulfuric acid as catalyst [128]. The yield of glucose reached 97% within 2 h, which was higher than that (86%) of the control group. The activation energy of maltose hydrolysis was greatly decreased from 126.4 to 97.9 kJ/mol with ultrasonic energy. For cellulose conversion, a sequential sono-assisted alkaline pretreatment (SAP) and sono-assisted acid hydrolysis (SAH) was proposed to selectively convert sugarcane bagasse into sugars [100]. Sono-assisted hydrolysis of the residue after pretreatment produced hydrolyzates with less inhibitor content (such as acetic acid, furfural), and favored the subsequent course for bio-ethanol production. The limited production of acetic acid (<1.0 *vs.* 3.65 g/l) and furfural (0.1 *vs.* 0.5 g/l) under ultrasonic irradiation primarily benefited from a lower acid hydrolysis temperature (50 *vs.* 122 °C) than conventional thermal hydrolysis, which provides clear process benefit and atom efficiency in the hydrolysis.

Direct application of ultrasound with enzymes accelerates saccharification and fermentation rate. The first-order rate constant of glucose fermentation over *S. cerevisiae* was enhanced by 1.3 times with sonication in a bath sonicator [129]. Similar results were observed with α-amylase [61,130,131]. The activation energy of α-amylase greatly decreases from 58.5 to 12.1 kJ/mol in an ultrasonic bath (40 kHz) [131]. Velmurugan and Muthukumar [102] tested sequential sono-assisted alkaline pretreatment (2% NaOH) and sono-assisted enzymatic saccharification over *Cellulomonas flavigena* (*MTCC 7450*), using sugarcane bagasse (SCB) as materials. The introduction of ultrasound accelerated the degradation of cellulosic materials to glucose, with the rate constant being remarkably promoted by 10–100% [78]. As a result, the glucose yield for a 6-h hydrolysis reaction was 66% higher than that for unsonicated saccharification [102].

Sono-assisted enzymatic conversion of cellulose can be performed in cellulose solvents such as ionic liquids and NMMO. Under sonication, the *in-situ* enzymatic hydrolysis of Avicel cellulose and sugarcane bagasse with a mixture of cellulase and β-glucosidase in 20% (*w*/*v*) aqueous NMMO solution gives conversion ratios of 95.9% and 90.4%, respectively, higher than the values in citrate buffer with conventional heating [132]. The relative activity of mixed enzymes show only a small decline after pre-incubation with NMMO concentrations not being higher than 20% (*w*/*v*).

The introduction of ultrasound not only enhances the conversion of cellulosic materials through intensified pretreatment, but also assists in the improvement of the efficiency in saccharification and fermentation. For ultrasound-assisted pretreatment, ultrasonic cavitation provides high-energy level that is sufficient for destroying cellulose structure. However, the enhancement of subsequent saccharification is mainly due to the sharp reduction of biomass size and the increase of cellulose hydrophilicity, while the change in chemical structure and crystalline structure of cellulose by ultrasound seems to be the minor contribution. For ultrasound-assisted saccharification and fermentation, the main reason for the enhanced conversion is the substantial improvement of mass transfer in reacting systems, as well as the activation of chemical and biological catalysts. Ultrasound accelerates the reaction kinetics of cellulose and hemicellulose toward fermentable sugars and bio-ethanol. The equilibrium yields of products such as fermentable sugars are generally increased. As the substantial limitation of contact of heterogeneous substrates with solvents and catalysts, the expected high equilibrium yield is not possible with conventional thermochemical methods. The enhanced solvation and the increased hydrophilicity/reactivity of biomass reactants by ultrasound is regarded to decrease the severity of thermochemical reactions, which means that reaction could take place at lower temperature within shorter time, with smaller requirement for corrosive acid or base catalysts. As a result, the product selectivity and atom efficiency can be greatly improved for many chemical systems.

3.3. *Fermentation of lignocellulosic wastes to biogas and bio-hydrogen*

Remarkable benefits were obtained for the production of methane and bio-hydrogen after ultrasound-assisted anaerobic or aerobic digestion was used. Many bioresources such as microbial sewage sludge [22,23,133], animal wastes (e.g., cattle manure) [134], fruit wastes [135], agricultural wastes [136], distillery residues [137] and by-products from bio-ethanol [138] have been tested.

The effect of ultrasonic energy was tested in the anaerobic fermentation of apple pomace to bio-hydrogen [135]. Apple pomace soaked in ammonia liquor and mixed with cellulase was ultrasonically treated for 20 min. H_2 yield after ultrasonic pretreatment increased from 55.5 to the maximum of 60.1 mmol/g total solid. Compared with other conventional physicochemical methods, anaerobic digester sludge showed remarkably higher bio-hydrogen production from glucose in batch reactors after ultrasonic pretreatment [139]. Pre-sonication of anaerobic sludge with temperature control (<30 °C) gave a relatively high hydrogen yield of 1.55 mol H_2/mol glucose, compared with that after HCl, heat-shock, NaOH pretreatment or without pretreatment, that had hydrogen yields of 1.11, 1.04, 0.68 and 0.70 mol H_2/mol glucose, respectively.

Ultrasonic energy can be directly integrated into the fermentation reactions. Sonication in a continuous stirred tank reactor gave average hydrogen production rates and hydrogen yields of 200–233 mL H_2/(L reactor-volume h) and 1.9–2.1 mol H_2/mol glucose, respectively, which were about 93% and 83% higher than conventional continuous stirred tank reactors [140].

3.4. *Mechanism and research needs*

Physical transient cavitation is the major cause for the ultrasound-enhanced treatments and reactions of lignocelluloses [97]. Considering the nonvolatility of lignocelluloses, the interaction of ultrasonic energy cannot take place in the high-intensity oscillating bubbles. The greatest possibility for energy transfer is through high-intensity physical effects such as shockwave, micro-convection or microjets, and these energy events happen in the bulk liquids and the solid/liquid interface. Therefore, the population and intensity of transient bubbles before collapse, as well as the properties of the bulk and muddy liquids, will have great influence on the processing performance.

The population and characteristics of cavitational bubbles depends on several key acoustic parameters, including frequency (commonly lower than 45 kHz), ultrasonic intensity and its distribution in reactors. However, ultrasonic power and sonication time should be selected or optimized according to the purpose for treatments. A power rating of 1–3 W/mL is normally for delignification and extraction of plant fractions such as polyphenols, flavones, alkaloids, terpenes, glycosides and waxes [16]. The chemical hydrolysis in laboratory is generally performed at 30–100 °C within 2 h, with rating power of 0.01–4 W/mL. Care should be taken for ultrasonic power input that is higher than 1 W/mL in the enzymatic hydrolysis or fermentation, as excessive strong cavitation tends to affect the activity and stability of biological cells and therefore enzyme lifetime and reuse [141]. For the preparation of micro/nanocelluloses, very high power ratings of 3–10 W/mL and dilute material concentrations of less than 1% tend to be required [86,88].

The physical and heterogeneous properties of the bulk and muddy circumstances should be carefully considered, including the size and concentration of solid particles in liquid, degassing/bubbling, liquid viscosity, the partitioning of the mixed but immiscible solvent. They not only determine the cavitation performance, but also absorb the released energy in the high-intensity secondary effects. The concentration of solid substrates in most studies is normally limited to 2–5 wt.%. If the concentration of solid substrates is increased to 5–10 wt.%, the efficacy of ultrasonic intensification decreases, and the conversion of solid lignocellulose requires higher power rating of ≥ 2 W/mL [83,116,118]. As a result, it is necessary to incorporate sonication with other auxiliary methods such as the addition of acid/base for processing the high solid concentration of biomass feedstock.

Although most of the studies were performed in laboratory, the scale-up of lignocellulosic pretreatment and reactions in larger scale reactor of up to several liters is possible. In a study on enzymatic saccharification of waste papers, benefits of ultrasonic intensification were also verified with single ultrasonic horn [78]. The introduction of sonication with same rating power (30 and 60 W) in larger reactors (3.2 and 6.4 L) improved the equilibrium yields of total sugars by 10–36%, depended on the different kind of waste paper. The kinetic constants increased by 15–90%. Compared to sonication in 0.8-L reactor, the ultrasonic intensification in these larger reactors was also encouraging. However, parameters such as specific power density were still suggested to be further optimized according to the reactor scale [78].

The following research needs are identified in this review for application of sonication to the pretreatment and reactions of lignocellulosic materials:

1) Incorporation of direct sonication with biological (such as fungus and enzymes) or chemical methods (homogeneous acid/base) for lignin digestion and lignocellulose conversion;
2) Combination of heterogeneous catalysts with ultrasonic intensification for the reactions of soluble reactants such as sugars [142,143];
3) Improvement of the performance and efficiency of ultrasonic cavitation by changing homogeneous or heterogeneous composition of lignocellulosic biomass solution, such as the addition of a second organic solvent or surfactant;

4) Reduction of energy consumption. High-energy consuming processes such as the decrystallization of cellulose can be avoided by parameter optimization and reactor design;
5) Analysis on the mechanism and kinetics levels with theoretical methods and simulation.

4. Ultrasound-assisted synthesis of biodiesel

Through catalytic esterification/transesterification with low molecular weight alcohols, the fatty glycerides and free fatty acids (FFAs) in lipids can be efficiently converted into monoesters (FAMEs) with high yields that simultaneously produce glycerol as by-product [4]. The obtained biodiesel, which contains long-chain FAMEs generally has a higher octane number, higher oxygen content, lower condensation point and a much higher flash point than conventional diesel, and therefore has better lubricity, security and biodegradability [4,5].

In biodiesel synthesis, there are three main reactions: (1) hydrolysis of fatty acid glycerides into FFAs, (2) esterification of FFAs into FAMEs and (3) transesterification of fatty acid glycerides to FAMEs with low carbon alcohols [4,5].

The critical problem for the catalytic esterification/transesterification is under heterogeneous conditions. The reactant FFAs or fatty glycerides is immiscible with low molecular alcohols and catalysts. Even worse is the high viscosity of reactants and products such as FFAs, fatty glyceride and glycerol. As a result, the equilibrium and kinetics of reactions are seriously influenced by the limits for the mixing or contact of oil with alcohols and catalysts, as well as the limits for the product transferring out. On the other hand, the development of biodiesel industry is more dependent on raw oils and catalysts. Nonedible and less expensive oils grown on leanness and harsh environments are suggested to replace conventional edible ones, while much high content of FFAs and impurities such as water should be removed before transesterification [144]. It is also an important issue for using solid acids/bases with high activity and stability to replace liquid alkali catalysts or sulfuric acid.

Ultrasonic energy plays two roles in biodiesel synthesis: (i) better dispersion, homogenization and emulsification of reactants and catalysts as the results of micro-convection and strong shockwaves generated by ultrasonic cavitation, and (ii) activation of chemical and biological catalysts. However, reaction mechanisms for catalytic esterification/transesterification do not change under ultrasonic conditions. This section discusses and reviews the progress of ultrasound-assisted synthesis of biodiesel including studies on edible and nonedible oils, strong acid/base, solid catalysts and biological enzymes. The intensification mechanisms are further addressed with some possible recommendations.

4.1. *Esterification over homogeneous/heterogeneous catalysts*

Esterification of medium- or long-chain FFAs is generally performed with homogeneous catalysts (such as H_2SO_4) under ultrasound assistance. The commonly used conditions are bath sonication, rating power density of <0.1 W/mL, alcohol/FFAs molar ratio of 3/1–9/1, H_2SO_4 mass concentration of 3–5%, temperature of 30–60 °C and time of 1–6 h. High yield of around 95% is achieved within much shorter time compared with that in the absence of ultrasound (Table 3) [145–147]. Catalytic esterification using glycerol instead of methanol or ethanol requires relative harsh condition with higher acoustic power density (2.8 W/mL) and higher temperature (70–90 °C) [80]. However, much shorter time from 24 to 6 h was also observed under ultrasonic condition for the esterification ratio of over 98%.

For crude oils with high content of FFAs, an additional esterification step is required before transesterification [148]. The required molar ratio of alcohol to oil, catalyst amount and reaction time are in the range of 2/1–5.5/1, 0.5–1.6% H_2SO_4 and 15–120 min, respectively, which depend on the acid values of crude oils (Table 3). With sonication, lower temperature (40 *vs.* 60 °C), shorter time (15 *vs.* 20 min) and less molar ratio of alcohol to oil (2/1 *vs.* 3/1) were enough for 89.6% reduction of the acid values in oils compared to conventional stirring [149]. This implies that esterification rate is greatly improved under ultrasonic irradiation [149,150]. Due to the reversibility of the esterification reaction, ultrasonic intensification could both affect the forward and reverse kinetics. However, the maximum apparent forward reaction rate constant (from FFAs to FAMEs) under ultrasonic condition was found much higher than that with stirring (0.034 *vs.* 0.004 min^{-1}) [150]. The reverse reaction rate constants under both conditions were almost zero, which meant the negligible contact of water with the formed esters in oils. For the esterification with ultrasound, the performance using bath-type reactor may be better than that with probe ones, possibly because of the relatively high and concentrated ultrasonic intensity in the probe-type reactor [62]. The pre-esterification of high acid-value *Nagchampa* oil with probe-type reactor seems even worse than that with conventional stirring [79].

For further intensification of esterification, a possible combination of ultrasonic energy with microwave was also proposed and adopted [79]. The sequential treatment under microwave and ultrasound achieved much better esterification results than that only with ultrasound. The optimized reaction time and the optimized alcohol/oil molar ratio decreased from 60 to 15 min and from 4/1 to 2/1, respectively. However, sequential treatment result with ultrasound and then microwave was inferior to the sequential treatment that used microwave first and then ultrasound.

Compared to homogeneous H_2SO_4, the catalytic esterification over heterogeneous acid required longer reaction time and higher amount of alcohols [151], while the esterification result strongly depends on the surface properties and performance of heterogeneous catalysts. The sonication of the mixture of FFAs (C8–C10) and methanol with 2 wt.% chlorosulfonic acid supported zirconium catalyst at 40 °C for 7 h reaches an equilibrium conversion of 97 mol.% [151]. However, serious deactivation of the catalyst was observed, with a conversion of 41 mol.% being for regenerated catalyst at the same reaction conditions.

Improved esterification at lower temperatures and shorter reaction times with less amount of alcohol solvent can be realized with relative low ultrasonic energy in bath-type reactor. The reversible acid catalyzed esterification is initiated by the protonation of carboxylic carbon atom by H^+ ions from the acids, and is terminated by removing a molecule of water. Therefore, the critical step is the timely removing of water from the products, which can be intensified by high-intensity ultrasound. The combination of ultrasound and heterogeneous superacid catalyst will give an improvement, however, the abscission of superacid group from the supporting catalyst in the sonication is a major challenge.

4.2. *Transesterification over homogeneous acids/bases*

To obtain a desired fuel quality with its range of carbon number within that of diesel fuel, it is necessary to transesterify the high molecular weight fatty acid triglyceride to FAMEs, which is commercially catalyzed by homogeneous acids or homogeneous bases such as NaOH or KOH or their methoxides. Although the transesterification is easily to be realized, the transesterification course is still to be intensified for solving the actually existence of mass transfer resistances from the immiscible reactants. With the help of ultrasonic cavitation, high biodiesel yields at relative lower temperature within much shorter time with less requirement for the corrosive base and alcohols used was achieved, compared with

Table 3
Ultrasound-assisted biodiesel synthesis.

Reaction	Materials	Catalyst	Reaction parameters[a]	Results	Advantages	Ref.
Hydrolysis/glycerolysis	Oil (edible, soy oil, olive oil) Water/glycerol	Enzyme: lipases such as *Novozym 435* [180]	Bath, 28–37 kHz, 0.5–1.7 W/cm^2, Or probe, 20 kHz, 3–5 W/mL [180], 30–65 °C, 2–6 h, Water/oil weight ratio of 0.5/1–2.5/1 [179], Or with surfactants (Triton X-100, AOT, Tween 65, Tween 85) [180]	1) Mono and diacylglycerols total yield, 40–55 wt.% [180], 2) Acid value of 50–70 mg KOH/g, with time reduced from 5–12 h to 1–5 h [179].	Larger water/oil interfacial area.	[179,180]
Esterification	Free fatty acids (oleic acid, palmitic acid, stearic acid, palm fatty acid distillate) Alcohols (methanol, ethanol, propanol, butanol)	Homogeneous: H$_2$SO$_4$ (3–5%)	Bath, 22–40 kHz, 0.04–0.07 W/mL, 30–60 °C, 1–6 h, Alcohol/acid molar ratio of 3/1–9/1	Conversion improved from 85–93% to 92–95% for FAMEs from palm fatty acid distillate (PFAD), from 65% to 75% for isopropyl esters from PFAD, with time reduced by 20–120 min [146,147].	Shorter reaction time.	[145–147]
	C8–C10 fatty acids (mainly caprylic acid and capric acid) Glycerol	Homogeneous: H$_2$SO$_4$ (5%)	Probe, 22.5 kHz, 2.8 W/mL 70–90 °C, 2–8 h, Glycerol/acid molar ratio of 3/1–9/1	High equilibrium conversion of 98.5% obtained with time shortened from 24 to 6 h.	Shorter reaction time.	[80]
	High acid-value nonedible oils (such as *Jatropha curcas* oil and *Nagchampa* oil) Methanol	Homogeneous: H$_2$SO$_4$ (0.5–1.6%)	Bath, 20–40 kHz, 0.04–0.9 W/mL, 30–50 °C, 15–120 min, Alcohol/oil molar ratio of 2/1–5.5/1	Second-order rate constant increased from 0.018 to 0.028–0.03 L/(mol min), with optimized temperature, time and molar ratio decreased from 60 to 40 °C, from 20 to15 min and from 3/1 to 2/1, respectively [149].	Biodiesel stabilized by removing free fatty acid. Reduced alcohol amount. Reduced temperature and time.	[149,150]
	Fatty acid odor cut (C8–C10), Methanol	Heterogeneous base: ClSO$_3^-$/Zr catalyst	Bath, 20 kHz, 0.035 W/mL, 40 °C, 3.5–7 h, Methanol/acid molar ratio of 10/1	FFAs' conversion, 75–97 mol.%	Less corrosive than H$_2$SO$_4$.	[151]
Transesterification	Oil (edible, soybean oil) Methanol	H$_2$SO$_4$ (5 wt.% of oil)	Probe, 20 kHz, 3 W/mL, 45–65 °C, 2–5 h, Methanol/oil molar ratio of 6/1	Biodiesel conversion of 69.2%.		[198]
	Oil (edible, soybean oil, rapeseed oil, vegetable oil, sunflower oil; nonedible, castor oil, waste cooking oil, pre-esterified *Jatropha* oil, pre-esterified *Nagchampa* oil) Alcohols (methanol, ethanol, propanol, butanol)	Homogeneous: NaOH or KOH (0.5–2.0 wt.%) Or 30% CH$_3$ONa in methanol	Bath/probe/plate, continuous/pulse, or with pilot apparatus, 20–45 kHz or 581–611 kHz [157], 0.02–8.7 W/mL, 25–60 °C, 15 s–60 min, Alcohol/oil molar ratio of 4/1–10/1	1) Best yield of 98–99% obtained at 28 kHz, while 40 kHz ultrasound is more effective in reducing time [158]. 2) Secondary and tertiary alcohols show less or no conversion [158]. 3) Second-order rate constant increased from 0.088–0.096 to 0.212–0.277 L/(mol min), with optimized temperature and time decreased from 65 to 40 °C, from 90 to40 min [149]		[54,148,149, 152–161]
	Oil (nonedible, untreated *Jatropha* oil) Methanol	Heterogeneous acid: γ-Al$_2$O$_3$ supported 25–35% H$_3$O$_{40}$PW$_{12}$	Probe, pulse, 20 kHz, 180–300 W, 65 °C, 30–50 min, Methanol/oil molar ratio of 19/1–24/1	1) Biodiesel yield of 83.6–89.3%. 2) Catalyst reused for 3 cycles with yield of >72.0%.		[178]
	Oil (edible, soybean oil, sunflower oil, rapeseed oil, palm oil; nonedible oil, pretreated *Jatropha* oil, waste soybean oil) Methanol	Heterogeneous base: CaO, SrO, BaO, mesoporous Na/SiO$_2$, mesoporous K/ZrO$_2$, Mg/MCM-41, Mg–Al hydrotalcites, et al.	Bath, 35–40 kHz, 0.02–0.06 W/mL, Or Probe, continuous/pulse, 24 kHz, 0.4–7 W/mL, 45–75 °C, 15–300 min, Methanol/oil molar ratio of 3/1–12/1	1) Biodiesel yield improved from 5.5% to 77.3% over CaO, from 48.2% to 95.2% over SrO, while catalyst is easy to be inactivated [168]. 2) Conversion of 96% over Mg–Al hydrotalcite, with time reduced from 20–24 h to 4–5 h, and catalyst reused for 8 cycles [159,170]. 3) Biodiesel yield improved from 61.0% to 75.6% with ultrasound within shorter time of 45 min, and even to	Less corrosive than homogeneous catalyst. Lower cost than enzyme.	[159,168–172]

				maximum value of 90–94% over microcrystalline CaO [169].		
	Oil (edible, soybean oil, coconut oil; nonedible, pre-esterified *Jatropha* oil, waste cooking oil) Methanol, ethanol, Dimethyl carbonate (DMC) [185]	Enzyme: immobilized lipase (*Novozym 435*, *Chromobacterium viscosum*, *Lipozyme RM IM*)	Bath, 25–43 kHz, 0.01–0.04 W/mL, 25–60 °C, 0.5–8 h, Alcohol/oil molar ratio of 3/1–6/1, Or DMC to oil ratio of 6/1 [185] Or with *tert*-amyl alcohol/oil volume ratio of 0.5–2% [182]	1) Methyl ester yield increased from 20% to 62% with ultrasound, to 96% with ultrasound and stirring for 4 h [182]. 2) Transesterification ratio over DMC increased from 38.7% to 57.7% with ultrasound, to 86.6% with ultrasound and stirring [185]. 3) Appropriate addition of *tert*-amyl alcohol improved the enzyme activity [182]. 4) Enzyme activity sharply declined after 4 cycles, even to zero with *Lipozyme RM IM* [183].	Catalyze both esterification and transesterification.	[181–185]
Pre-irradiation of enzyme	*Jatropha* oil	Enzyme: Lipase from *Burkholderia cepacia* (PS), *Pseudomonas fluorescens* (AK)	Bath, 40 kHz, about 0.02–0.04 W/mL, 40 °C, 2–4 h (pre-irradiation) + 24 h (transesterification), Ethanol/oil ratio of 1.37/6 (*v*/*v*)	Transesterification ratio increased from 34% to 79% for *Jatropha* oil.	Transesterification efficiency showed improvement	[186]
Pre-emulsification	Palm oil Water	No	Probe, 20 kHz, about 2 W/mL, 1 min, With acetate buffer and emulsifier Gum Arabic Then hydrolysis at 45 °C	Initial hydrolysis rate over solid lipase from *Candida rugosa* and liquid lipase from *Mucor miehei* improved from about 0.1–0.65 to about 0.35–1.95 mol/(m^3 min)		[196]
	Soybean oil, Ethanol	$Ti(Pr)_4/Al(Pr)_3$	Probe, pulse (100% cycle), 24 KHz, 0.37 W/mL, 60 °C, 470 s, Alcohol/oil molar ratio of 6/1, Then 150 °C, 2 h, without ultrasonic	Transesterification ratio increased from 2–48% to 18–64%.	Quasi homogeneous reaction, better mass transfer.	[166]
	Oil (soybean oil, rapeseed oil, palm oil), Alcohols (methanol, ethanol)	No	Emulsifier/bath, Then 300–350 °C, 20–30 MPa, 10–16 min, without ultrasonic, Oil concentration of 10–80 vol% Or 250–280 °C, 6–12 min, without ultrasonic, methanol/oil molar ratio of 40/1–50/1 [190].	1) Conversion ratio at 300–310 °C increased from 47–68% to nearly 85% [189]. 2) Supercritical temperature for transesterification decreased from 350 °C to 265.7 °C [190].	Decreased temperature. Shorter time for good product yield. Low ethanol/oil molar ratio of about 4/1 acceptable for supercritical transesterification [189].	[189,190]

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[a] Values given for acoustic energy used are power ratings.

conventional mechanical stirring or even hydrodynamic cavitation [148,149,152–154]. In Table 3, the acoustic intensification happens in a bath-, probe- or plate-type reactor at frequency of 20–45 kHz and general rating power density of 0.02–3 W/mL, with the processings in laboratorial or pilot scale [54,148,149,152–161]. Some nonedible oils with high FFAs (e.g., *Jatropha* oil and *Nagchampa* oil) should be pre-esterified, as their high initial acid value may result in troublesome saponification in direct base-catalyzed transesterification [148,149]. The suggested catalyst amount and the alcohol/oil molar ratio were 0.5–2.0 wt.% and 4/1–8/1, respectively.

Generally speaking, the thermodynamic equilibrium in the homogeneous transesterification does not change with the introduction of ultrasound, as the final biodiesel yields (96–100%) in most cases have little variance. However, as the intensified mass transfer of the reversible reactions, a new kinetic relationship may be established for quicker achievement to this equilibrium yield. For base-catalyzed transesterification, there are three reversible elemental steps [162,163]

$$\text{Triglycerides} + CH_3OH \rightarrow \text{Diglycerides} + \text{FAMEs} \qquad (1)$$

$$\text{Diglycerides} + CH_3OH \rightarrow \text{Monoglycerides} + \text{FAMEs} \qquad (2)$$

$$\text{Monoglycerides} + CH_3OH \rightarrow \text{Glycerol} + \text{FAMEs} \qquad (3)$$

With mechanical agitation, the second step is the rate determining step, while for ultrasound-assisted transesterification, the transformation of diglycerides to monoglycerides is greatly enhanced so that the third step becomes the rate determining step [163]. Gole and Gogate [149] reported similar results with the remarkable increase of second-order rate constant under ultrasonic irradiation. However, the transesterification is still hard with secondary or tertiary alcohols such as *iso*-propanol, *iso*-butanol and *tert*-butanol, which is attributed to their steric hindrance, even with ultrasonic intensification [158,164].

In most cases, the transesterification with relative higher ultrasonic frequency (40–45 kHz) is reported within shorter reaction time than those with lower frequency (25–28 kHz), which asks for less requirement of energy consumption [154,158]. Mahamuni and Adewuyi [157,165] found that using an even higher frequency (581 or 611 kHz) achieved better results for KOH-catalyzed transesterification of soybean oil. The further improvement on the transesterification efficiency with the introduction of multifrequency seemed also possible.

The combination of ultrasound with mechanical stirring can further intensify the mass transfer in the base-catalyzed transesterification. For sunflower oil, the combination of flat plate sonication with stirring achieved similar equilibrium yield within much shorter time (25 *vs.* 50 min) with less methanol/oil molar ratio (5/1 *vs.* 6/1) and less NaOH concentration (1.0 *vs.* 1.5 wt.%), compared to that with sonication only [155]. As a result, excess energy requirement for long time processing with high-intensity ultrasound was saved.

The introduction of emulsifying agent is another alternative solution to further enhance the emulsification of the immiscible reactants. By ultrasound assistance, alcohol/oil mixtures can be emulsified with metal *iso*-propoxides (e.g., $Ti(Pr)_4$ and $Al(Pr)_3$), which are used both as surfactants and catalysts for quasi homogeneous catalytic transesterifications [166]. Partially polymerized titanium *iso*-propoxides $Ti(Pr)_4$ showed better catalytic activity than $Al(Pr)_3$, with biodiesel yield in the ethanol/soybean oil/$Ti(Pr)_4$ nanoemulsions significantly increasing from 48% without ultrasound to 64% under ultrasonic irradiation.

Combination of sonication with microwave irradiation is also studied for the base-catalyzed transesterification [79,167]. The sequential application of microwave (160 W) and ultrasound (120 W) in 250 mL three-necked bottle obtained a similar equilibrium transesterification ratio of high acid-value *Nagchampa* oil within shorter time (6 *vs.* 20 min), with less methanol/oil molar ratio (4/1 *vs.* 6/1), compared with sonication only [79]. Similar intensification is also seen in the work of Hsiao et al. [167]. However, the treatment order is first with ultrasound then with microwave [167]. With 1-min ultrasonic mixing and then 2-min closed microwave irradiation, the conversion rate of soybean oil to biodiesel was dramatically increased to 97.7%, which was much higher than that with sonication for 5 min only (72.5 wt.%) or with microwave for 10 min only (21.0 wt.%).

Because of higher biodiesel yield and less impurities (such as unreacted alcohols) remaining in the products, the obtained biodiesel under ultrasonic conditions may have higher kinematic viscosity, lower flash point and less acid content than that from conventional methods [149]. Biodiesel products from some nonedible oils may have higher density and higher dynamic viscosity, and cannot be directly used in the diesel engines, because of the different composition of fatty acid triglyceride in the oil materials [154]. However, the quality of the obtained biodiesel could be further updated by the ultrasound-assisted conversion of blended mixtures of nonedible oils with edible ones at certain ratios [161].

The introduction of ultrasound does not change the chemical mechanism of homogeneous base-catalyzed transesterification. However, sonication accelerates the kinetics of limited steps in the conversion, and thus remarkably improved the efficiency of overall reaction. Therefore, the time required for transesterification is greatly reduced by 50–80%. Good transesterification results can also be achieved at lower temperature with ultrasound, as ultrasonic cavitation gives additional energy to the reactants that counteracts the detrimental effects of decreased temperature on the reaction kinetics. The increase of ultrasonic frequency from 25 kHz to higher frequency benefits the improvement of intensification performance and energy economics in ultrasound-assisted transesterification, as higher frequency ultrasound increases the energy intensity of active cavitation. The combination of sonication with other methods such as mechanical stirring and microwave achieves better transesterification with lower consumption of ultrasonic energy, which further improves the processing benefit in ultrasound-assisted transesterifications.

4.3. *Transesterification over heterogeneous acids/bases*

Solid catalysts provide a green and promising way for biodiesel synthesis, with less corrosion than homogeneous acid/base catalysts and simplified recovery of the catalysts. The available heterogeneous catalysts for base-catalyzed transesterification including single or composite metal oxides (CaO [168,169], SrO [168], BaO [168], $ZnAl_2O_4$ and calcined Mg–Al hydrotalcites [159,170,171]), supporting alkaline catalysts (Na/SiO_2 [172], KF/CaO [173], KF/Al_2O_3 [174], microporous titanosilicate ETS-10 (Na, K) [175] and molecular sieves (e.g., Mg/MCM-41 [159,171]), have been studied under ultrasonic conditions. As concluded in Table 3, the heterogeneous catalysis of transesterification could be performed in an ultrasonic bath (0.02–0.06 W/mL), or with a high-intensity sonotrode (0.4–7 W/mL). The general range of methanol/oil molar ratio, catalyst amount and reaction time were 3/1–12/1, 0.5–12.5% and 15–300 min, respectively. However, all these actual parameters for ultrasonic operation strongly depend on the properties of the catalysts used. The raw oils for base-catalyzed reactions could be nonedible oils (e.g., *Jatropha* oil and frying soybean oil), while in most cases, they are esterified or pre-refined to remove excess FFAs of over 3% before the formal reactions [170–172].

However, a recent case conducted the ultrasound-assisted transesterification of untreated castor oil and *Jatropha* oil in methanol–chloroform (1:2 *v*/*v*) over SrO catalyst, and obtained a relative high conversion to FAMEs (92.1–93.6%). They also used broken seeds as the raw materials without any further pretreatment, while similar biodiesel yields were achieved [176].

For base catalysts, the transesterification activity might depend on the basic strength of the solid catalysts. Excess basic strength was necessary to shift the reversible esterification and transesterification forwards. For example, alkaline earth metal oxide BaO was believed with stronger basic strength than SrO and CaO, and accordingly exhibited a better activity in the catalytic transesterification of palm oil [168]. However, notable leaching of BaO into the reaction mixtures seriously limits its recycle and reusability [168]. Therefore, high basic strength of catalysts does not mean the high stability and acceptability. The deactivation of heterogeneous catalysts should be persistently considered, while most heterogeneous base catalysts after ultrasonic reactions could be reused for 3–8 cycles.

Ultrasonic energy indeed intensifies the transesterification over solid base catalysts. First, high equilibrium yield of biodiesel (up to 89–98%) was obtained within substantially shortened time [159,169,171]. Secondly, the number of reusing runs for these heterogeneous catalysts increased. For the conversion of sunflower oil to FAMEs over microcrystalline CaO, biodiesel yield sharply decreased from 61.0% to 34.4% at the second recycling run with conventional stirring and thermal activation, while it remained high biodiesel yield (>74%) in the third reusing run of the catalyst under ultrasound [169]. The result could be due to two aspects: 1) viscous by-products such as glycerol are absorbed on the surface of the catalyst, and blocks the activate sites, while ultrasonic vibration helps in cleaning them timely during the reactions [170]; 2) the assistance of ultrasound reduces catalyst leaching into biodiesel layer, possibly because the reaction time is shortened [168]. As a result, the obtained biodiesel with ultrasonic intensification could have better quality accordance with the international standards than conventional methods, with less impurities (FFAs, sulfated ash and moisture), higher flash point and a better clarity [177].

On the other hand, solid superacids such as (Nafion, SO_x/ZrO_2, WO_x/ZrO_2) have also proven their effectiveness in conventional transesterification reactions [175]. Badday et al. [178] used γ-Al_2O_3 supported $H_3O_{40}PW_{12}$, which was regarded as a strong solid heteropolyacid catalyst, to catalyze the transesterification of crude *Jatropha* oil to their methyl esters under the action of probe ultrasound in pulse mode. The reaction had two attracting peculiarities than other reactions: first, it could directly catalyze the crude *Jatropha* oil with the initial content of FFAs over 10 wt.%, while no flocs precipitation or quality deterioration of the obtained biodiesel was observed, which was normally happened in the catalytic transesterification with homogeneous H_2SO_4 [148]. Second, for the case, a much higher molar ratio of methanol to oil was needed, which was 19/1–24/1 determined by experimental optimization. The author reported a relative high biodiesel yield of 83.6–89.3% over this heteropolyacid catalyst, while the catalyst could be reused for 3 cycles, as the stronger absorption of phosphotungstic acid on γ-Al_2O_3 than ZrO_2 or SiO_2 benefited the minimal leaching of active compositions [178].

Sonication intensifies the mass transfer in the heterogeneous catalyzed transesterification, and helps in the timely removal of viscous by-products. As a result, the transesterification rate is promoted, and the equilibrium yield of FAMEs also increases. Although high-intensity ultrasound has some influence on the structure and activity of heterogeneous catalysts, the stabilization of catalyst activity in reactions is mainly determined by the properties of the prepared catalysts. Catalysts that are stable in thermally-driven reactions tend to be stable in the presence of ultrasound. The integration of solid acid catalysts with ultrasound shows great potential on the treatment of high acid-value nonedible oils, since they can be used in the esterification and transesterification of high acid-value oils in one step. Nevertheless, the stability of heterogeneous acid catalysts is a topic that needs to be addressed.

4.4. Enzyme-catalyzed hydrolysis/esterification/transesterification

Compared to chemical catalysts, lipases can catalyze hydrolysis, esterification and transesterification reaction in one pot (Table 3) [4]. Hydrolysis or glycerolysis of plant oil to FFAs catalyzed by lipases such as *Novozym 435* was visibly accelerated and improved in an ultrasonic bath [179,180]. For ultrasound-assisted enzymatic transesterification, the commonly adopted conditions are bath sonication (<0.1 W/mL), alcohol/oil molar ratio of 3/1–6/1, reaction temperature of 25–70 °C, time around 4 h and catalyst amount of 1–10 wt.%, respectively (Table 3) [181–184]. Edible and pre-esterified nonedible oils including *Jatropha* oil, waste cooking oil were used, while excess water should be removed from raw oils to avoid the detriment influence on lipase activity [182]. Methanol or ethanol is the commonly used alcohols, while dimethyl carbonate as a possible alternative was also suggested [185]. The available lipases could be immobilized *Novozym 435*, *Chromobacterium viscosum* and *Lipozyme RM IM*, with the commercial *Novozym 435* being preferred [181–184]. With sonication, considerable transesterification yield of 85–96% was achieved within 3 h, while for conventional methods, much longer time (about 50 h) is needed for a yield of 80% [184]. Esterification is also possible with lipases. Esterification rate might be 1.5–2.5 times quicker than the transesterification rate with raw materials containing both triglycerides and FFAs [184]. Additionally, in most ultrasound-assisted enzymatic reactions, molar ratio of alcohols to oils is as low as only 3/1, just the stoichiometric ratio for transesterification, which means the least waste of unreacted alcohol was produced [182–184].

Ultrasonic intensification comes from two aspects: one is from the physical intensification of mass transfer, while the other is the activation of lipase itself. Lipases after ultrasonic pre-irradiation may have higher activities. The pre-irradiation of lipases (*Burkholderia cepacia*) in an ultrasonic bath for 3 h affected the tertiary structure and the microenvironment of the aromatic amino acids of lipases [186]. As a result, the transesterification rate of ethyl butyrate to butyl butyrate and *Jatropha* oil to biodiesel over the pre-irradiated lipases increased from 66% to 82% and from 34% to 79%, respectively [186].

The combination of ultrasonic irradiation and mechanical vibration intensified the reactions as compared with ultrasound only. The transesterification of soybean oil to methyl esters over immobilized *Novozym 435* achieved a yield of 96% with both ultrasound and stirring in 4 h reaction time, compared to that with stirring only (20%) or ultrasound only (62%) [182]. Similar results were also found with dimethyl carbonate [185].

Improved conversions of edible oil and nonedible oil can be achieved in one pot with lipases and sonication, as lipase has higher tolerance to high FFAs content than acid/base catalysts. The sonication can be directly integrated into the enzymatic reactions, or is used in the pre-irradiation of enzymes to stimulate the activity of these biological catalysts. The reaction conditions including temperature, irradiation time, ultrasonic intensity and frequency should be elaborately optimized, and provide the best circumstance for the catalysis of lipases, however, the optimized parameters are mainly determined by the properties of lipases themselves under the combined action of sonication, heating and solvents.

4.5. Ultrasonic pretreatment in supercritical synthesis of biodiesel

Transesterification of lipids is also carried out using supercritical methanol or ethanol. The supercritical synthesis of biodiesel is regarded as a green technology, as it needs less reaction time without catalysts such as corrosive sulfuric acid [187,188]. However, the necessity for harsh conditions including temperature of over 350 °C, pressure of over 20 MPa and high alcohol/oil molar ratio of generally 40/1–50/1, makes this course uneconomic and difficult for scale production. Low-frequency ultrasonic pretreatment helps in the formation of a relative stable emulsion, and overcomes the initial mass transfer resistance in subsequent supercritical reactions. First, the pre-sonication of palm oil with methanol and ethanol greatly improved the conversion rate of batch and continuous transesterification to nearly 85%, compared with 47–68% for reaction without pretreatment [189]. Second, considerable biodiesel yield was obtained at relative mild temperature of 280–310 °C [189,190]. Third, high biodiesel yield was obtained with much less alcohol usage. By using pre-sonication, Biktashev et al. [189] obtained biodiesel yield of about 82% at 300 °C and retention time of 10 min with the molar ratio of ethanol to oil of about 4/1. It is comparable or even superior to the molar ratio (3/1–12/1) at ambient conditions, and is much less than that needed for conventional supercritical transesterification.

4.6. Ultrasound-assisted conversion of glycerol – a by-product of biodiesel

Glycerol can be used as an industrial feedstock for many value-added chemicals such as polyols, triacetin and alkyd resins. It is also important additive for pharmaceuticals, cosmetics and foods. One of the great and green sources for the production of glycerol is from the biodiesel industry [191]. The transesterification of oils with methanol generates about 10 wt.% of glycerol as the main by-product [192]. Therefore, the utilization of spent glycerol influences the efficiency and economics of the integral biodiesel production. The improvement of thermochemical/biochemical conversion of glycerol and its derivatives was reported with ultrasonic intensification. Khanna et al. [193,194] ultrasonically enhanced the bioconversion of glycerol to 1,3-propanediol and ethanol over free or immobilized *Clostridium pasteurianum*. By using ultrasonic bath, the molar yield of 1,3-propanediol increased from 20.2% to 27.5% over immobilized enzymes with initial glycerol concentration of 10 g/L. However, in the case of free enzymes, ethanol is the most abundant product with low molar yield of 2–12%, and the yield increased by 55–83% under ultrasonic irradiation. The simulation of bubbles dynamics showed that the active cavitational bubbles were dominantly stable bubbles, not transient ones, which avoid the possible deteriorative influence on the biological cells [193]. Therefore, the oscillatory micro-convection and mild shockwaves of about 10 kPa would become the main intensification mechanism. The results also demonstrated that the behaviors of enzymes (enzyme activation, as well as the enhanced desorption of CO_2 and inhibitor from enzymes) might be the limited factor, as the increase of glycerol conversion and the significant variance of product selectivity happened at the almost unaltered uptake of glycerol by enzymes with ultrasound [193]. In another work, Bolívar-Diaz et al. [195] found that the activation in an ultrasound bath enhanced the conversion and selectivity of solvent-free transformation of glycerol carbonate (glycerol derivative) to glycidol over solid ZnO-supported nano-Co_3O_4 catalyst at more moderate temperature (60 *vs.* 150 °C) within shorter reaction time (7 *vs.* 23 h).

4.7. Mechanism and research needs

Many reports attribute the acceleration of biodiesel synthesis under ultrasound to the dispersion of the heterogeneous phase by the physical effects of ultrasonic cavitation [189,196,197]. Cavitation in the ultrasonic field produces very strong shear forces on liquid reactants (oil/lipids + light alcohols), breaks two-phase solutions into tiny droplets, and then intensifies the mixing and emulsification. The enhanced emulsification increases the original interfacial boundary of the immiscible binary mixture, which results in reduced interfacial contact resistance and improved mass transfer [57,198].

The size distribution of the droplets that are generated by ultrasonic irradiation have been studied for lipid–methanol systems [197,199] and for lipid–water systems [196]. Sonication (0.013 W/mL) produced dispersions with an average droplet size of 42% smaller than that with conventional impeller agitation in methanol/soybean oil systems [197] (Fig. 2a). Factors such as sonication power and input energy density influence the droplet sizes [197]. As sonication power increased from 0.013 to 0.031 W/mL (input energy at 90 J/mL), the mean droplet size reduced from 156 to 146 nm. The mean droplet size decreased from 360 to about 140 nm as the input energy density increased from 10 to 50 J/mL, and then became a constant at higher energy levels. The emulsification was further intensified with some emulsifying agents such as multi-bond metal *iso*-propoxides [$Al(Pr)_3$ or $Ti(Pr)_4$], which produces transparent alcohol/oil emulsions in 4 min sonication, with micelle sizes being 5.1 nm and large interfacial areas (Fig. 2b) [166].

However, the ultrasonic intensification is not merely through the enhanced contact of immiscible liquid phase. It also includes the timely disengagement of products and by-products such as glycerol and water from the local reaction system. Therefore, a new and quick equilibrium is established between the forward kinetics and the reverse ones in the reversible reactions, which brings higher final biodiesel yield within shorter time.

In fact, ultrasonic intensification does not change the chemical mechanism. In base catalysis, reactions are initiated by the generation of alcohol anions under the attacking of OH^- ions from alkaline catalysts, while the first and critical step in acid catalysis is the protonation of triglyceride directly by H^+ ions from the acids. Therefore, a relative high methanol/oil molar ratio benefits the formation of methoxides for base catalysis, while it is unfavorable to acid catalysis, as the concentration of H^+ ions in methanol may be diluted [198]. The intensification of chemical reactions under sonication is also the combined result of chemical activation and ultrasonic cavitation. In the catalytic transesterification of soybean oil over H_2SO_4, the rate constant of reactions at 25 °C ($1.0–1.2 \times 10^{-4}$ min^{-1}) was smaller than that at 15 °C ($3.6–3.7 \times 10^{-4}$ min^{-1}), indicating that the influence from ultrasonic cavitation overwhelmed that from the decrease of activation energy at relative higher temperature. When temperature is higher than 25 °C, the decrease of activation energy at the elevated temperature contributed to the dominated influences [198]. Similar situation is for the alcohol/oil molar ratio: lower ratio benefited the ultrasonic cavitation, and higher ratio favored the forward kinetics of the transesterification reactions toward products [198]. On the other hand, for the reaction systems with solid catalysts and enzymes, the adsorption of reactants on the active sites of the catalysts and the desorption of products, as well as the activation and deactivation of catalysts, should also be carefully considered.

As a whole, the intensification of ultrasound in biodiesel synthesis is physical. The chemical species generated from alcohols within the high-intensity cavitational bubbles are gaseous alkanes, H_2 and CO_2, not alcohol anions, which are the critical intermediates for attacking triglyceride molecules and initiating the transesterification reaction [200]. Therefore, the degree of ultrasonic

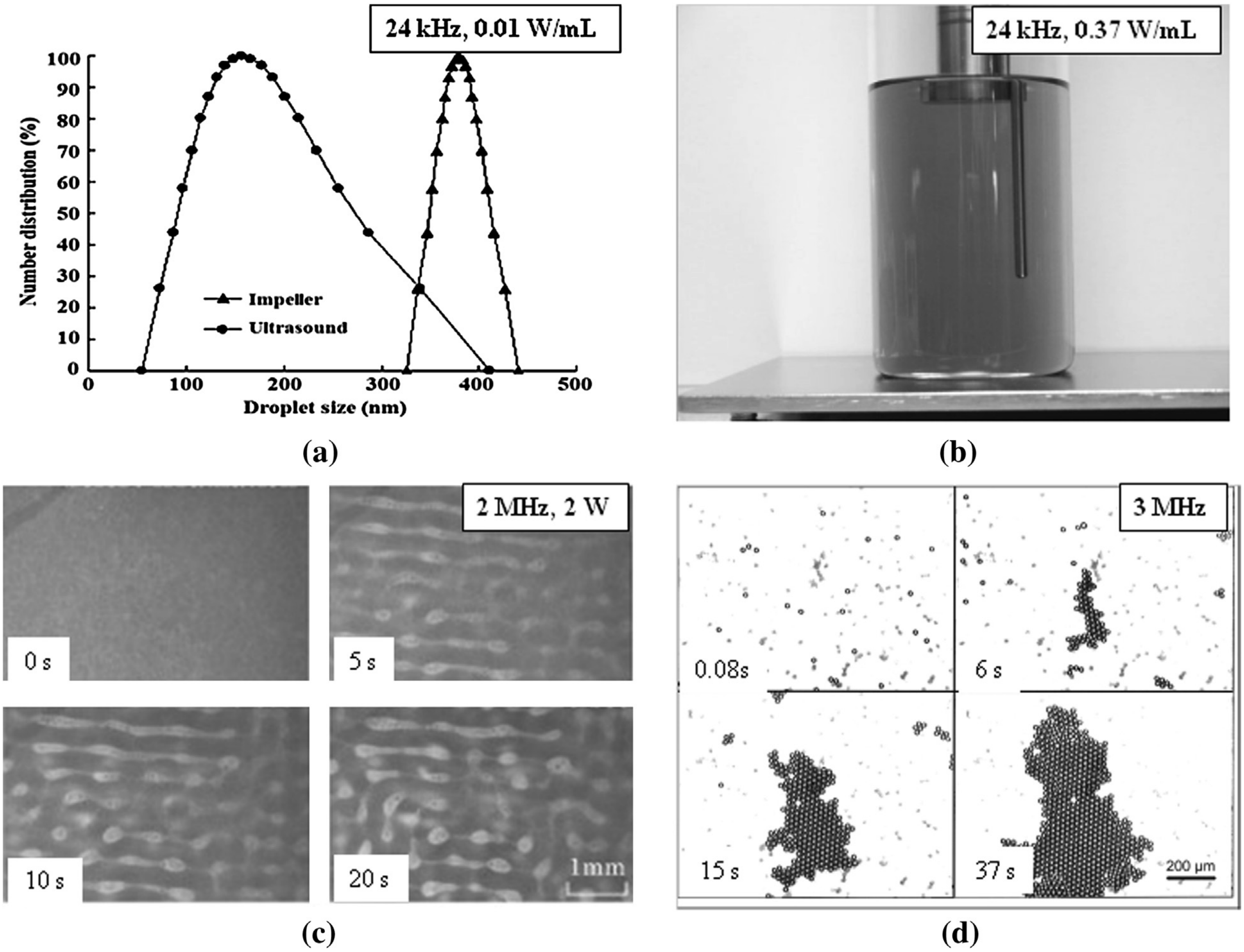

(a) (b) (c) (d)

Fig. 2. Emulsification, demulsification and agglomeration with ultrasonic energy. (a: Comparison of droplet size for impeller and ultrasonic agitation with input energy of 10.8 J/mL. Reprinted with permission from Ref. [197], Copyright ©2007 Springer. b: Stable nanoemulsion formed in ethanol/soybean oil/Ti(Pr)$_4$ mixture. Reprinted with permission from Ref. [166], Copyright ©2007 John Wiley & Sons, Inc. c: Demulsification and flocculation in canola oil–water emulsion. Reprinted with permission from Ref. [247], Copyright ©2009 Elsevier. d: Aggregate development of 25-μm white latex particles with times, Reprinted with permission from Ref. [293], Copyright ©2003 John Wiley & Sons, Inc.)

intensification may be primarily attributed to the violence of micro-convection generated from the collapse of active bubbles. Due to viscous effect, the cavitation behaviors in oils are much weaker than that in alcohols. So, in oil phase, there was less population of active bubbles, less violence in the radial motion of the bubbles (bubble radius expansion ratio of 2 *vs.* 5) and thus less intensive micro-convection (0.3 *vs.* 2.1 cm/s) [200]. For the reactant system with less amount of alcohol (alcohol/oil molar ratio of 6/1), the dispersion of alcohol in the oil determines the interfacial area for reactions. It is easy to obtain a uniform dispersion because of the remarkable cavitation in alcohol. However, with a higher alcohol/oil molar ratio (24/1), it needs to break up the viscous oil and disperse it in abundant alcohol, and of course the problem of low dispersion becomes pronounced [200]. Such intensification is also proved by Hingu et al. [160]. The position of the ultrasonic horn had great influence on the conversion in the KOH-catalyzed transesterification of waste cooking oil, which is 89.5% and 58.5% when the horn tip is placed at methanol rich layer and the interface between oil and methanol phases, respectively, while it is only 8.5% with horn tip deeply digging in the oil phase.

The following points are recommended and identified in this review for ultrasound-assisted biodiesel synthesis:

1) Match the choice of sonochemical reactors and acoustic parameters to the purpose and actual chemical mechanism. In homogeneous reactions, pre-emulsification with less ultrasonic energy may ensure sufficient contact of reactants and catalysts for subsequent reactions.
2) The advancement of reactor geometry, transducer design (such as the choice of the diameters of the horn tip), transducer placement (the depth of tip in the solution, the placement of transducer at bottom or side place, transducer array for scaled processing) and operation parameters (such as using pulse ultrasound and ultrasound with relative high frequency or even multifrequency).
3) Studies on the preparation and application of solid catalysts and enzymes that have higher activity and higher stability under sonication. The solid catalysts and enzymes could be considered in the one-pot catalytic reactions of nonedible oils.
4) Combined intensification of ultrasonic cavitation with other technologies such as microwave, mechanical mixing and surfactant addition.
5) Timely separation of by-products such as glycerol by flow processes.

5. Ultrasonic enhancement of algae pretreatment and reactions for biofuels

As an emerging bioresource, algae is attracting attention as a favorable bioresource, since it has special advantages for use in third generation biofuels [6] and chemicals [7]. First, algae have high photosynthesis efficiency. They grow quickly and have a short growth cycle compared with wood or grass. Their annual biomass productivity per hectare of land is approximately tenfold greater than that of terrestrial plants [201]. Second, compared with agro-biomass, algal cells have a simpler structure that allows them to be crushed at relatively mild conditions, and to be fractionated, extracted or converted to biofuels with less power consumption than other types of biomass. Third and most importantly, algae cultivation needs less land resources [202]. Generally, according to the cellular morphology, algae can be divided into three categories: cyanobacteria (a prokaryote), microalgae (visible with the aid of a microscope) and macroalgae (visible to the naked eye, mainly referred to as seaweed) [7]. Many kinds of microalgae like *Chlorella*, *Dunaliella salina* and diatoms have the ability to accumulate lipids in their cells, and are therefore believed to be promising materials for biodiesel synthesis [201,202]. Other algae such as cyanobacteria, macroalgae and some subgenus of microalgae contain a high content of starch, polysaccharides, oligomers and even hydrocarbons, and are more suitable as feedstocks for the production of chemicals such as sugars, bio-alcohols, H_2 and biogas (methane) through thermochemical or biochemical routes [7].

A general process for the production of biofuels from algae (microalgae and macroalgae) is shown in Fig. 3 with following steps:

1) Large-scale cultivation of algae. The main difficulty comes from the stringent requirements for nutrient source and surroundings for the cultivation of algae, which results in high cost. Quick growth of algae within shorter life cycles, as well as the improvement of the concentration of useful compositions in the algal cells is also expected;
2) Harvesting algae from nutrient water. This includes concentration (primarily for microalgae), separation and dehydration. The enrichment and dewatering of algae are very difficult with high-energy consumption. The raw algae suspension should be concentrated by at least 14–50 times to reach algae concentration of 1%, and meet the minimum demand for the subsequent conversion [7]. Therefore, advanced intensification technologies are needed to reduce the much high-energy demand.
3) Extraction of chemicals from algae and thermochemical/biochemical conversion. This mainly includes algae pretreatment such as the rupture of algal cells, extraction of polysaccharides and lipids, conversion of carbohydrate components to sugars, bio-ethanol and H_2, and conversion of oil fractions and fatty acids to biodiesel. The effective utilization of algae resource is still a novel and developing technology, and therefore is filled with many uncertainties.

In this section, the influence of ultrasonic energy on the intensified pretreatment and conversion of algae is discussed. It shows that ultrasonic energy notably accelerates the agglomeration, dewatering and disruption of microalgal cells, and facilitates its subsequent extraction and reaction.

5.1. Microalgae growth and nutrient accumulation

Ultrasound with low intensity and dose enhances the capability of assimilating and utilizing nutrients in heterotrophic cells, and improves their biomass productivity, physiological activities and

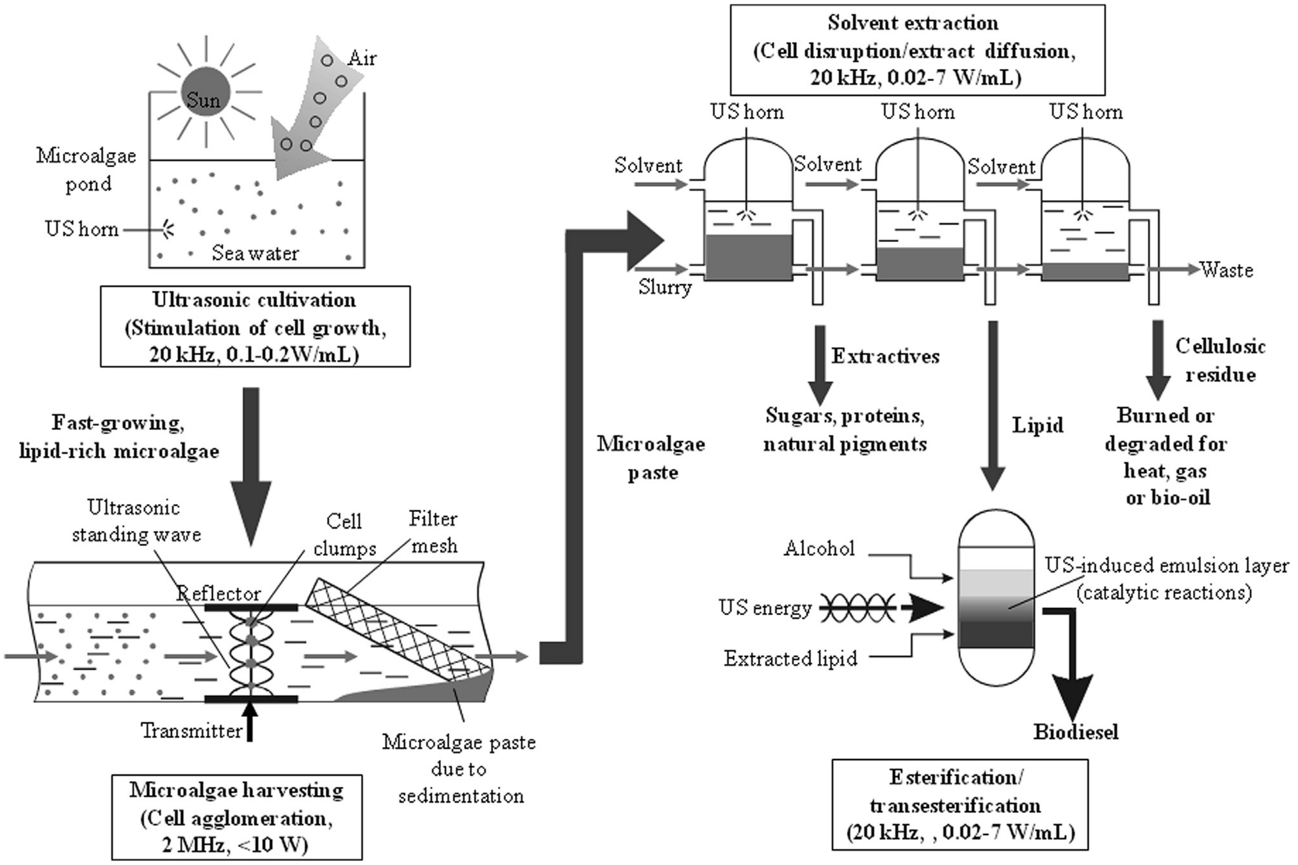


Fig. 3. Ultrasonic-assisted (US) processes for biodiesel from microalgae [31,206,208,237].

the contents of valuable components such as proteins and lipids [203].

At the end of the last century, many researchers studied the improvement of the productivity and protein content in some limnetic algae like *Scenedesmus acutus*, *Spirulina maxima*, *Anabaena flos-aquae* and *Selenastrum capricornutum* under low-dose ultrasonic irradiation [203–205]. Bozhkova and Dencheva [204] reported that increased biomass growth yield (by 9–11%) and increased protein content (by 5–10%) could be achieved with ultrasonic pretreatment of *S. acutus*. However, for *S. maxima*, sonication (20 kHz, 0.14–0.16 W/mL) for total duration of 20–40 s in 7–14 days notably inhibited the cultivation of *S. maxima*, suppressed algae growth by about 3–17%, and thus reduced protein content by 47–60% [205].

Sonication is reported to be beneficial for the accumulation of chemicals in the microbial body. The stimulation of sewage sludge with pulse ultrasound at low power of 0.28 W/g sludge favored the activation and growth of biological cells, and thus enhanced the accumulation of lipids by *Lipomyces starkeyi* when grown on sewage sludge [206]. Although no other corresponding reports have been published, the potential of ultrasound-stimulated accumulation of valuable chemicals such as lipids, starches and polysaccharides in microalgal cells is believed to be possible, just as for the case of yeast. The performance of ultrasonic stimulation might be related to the species of algae, while the metabolic interrelationship among nutrients in algal cells, such as the relationship of lipid formation to that of starch, may be affected [7].

5.2. *Harvesting of microalgae*

Unlike macroalgae, microalgal cells are generally suspended in water and are difficult to be harvested because of their small size (1–100 μm). Special techniques are necessary to harvest the cells such as chemical methods, which require chemical reagents and post-separation steps that may lead to contamination [207]. Alternatively, acoustic technology using standing waves has proven to be effective for harvesting and dewatering microalgal cells [208,209]. Sonication has already been widely used for the removal of detrimental algae such as cyanobacterial blooms from water in pools, lakes and rivers [210,211].

For harvesting microalgae, ultrasound-enhanced aggregation followed by gravitational sedimentation is generally used [208]. In the ultrasonic aggregation step, ultrasonic equipment consisting of a transducer and a reflector is used (Fig. 3). The distance between the transducer and the reflector is adjustable and the arrangement should be finally set to be an integral multiple of half-wavelength of ultrasound transmitted (length $= n\lambda/2$, $n = 1,2,3,\ldots\ldots$) (Fig. 3) in the solution being treated. When the equipment is turned on, the standing waves created numerous points that have strong vibrancy (antinodes) and others with nearly zero amplitude (nodes) in the reaction chamber (Fig. 3). Ultrasonic waves between transducer and reflector exert forces on microalgal cell particles and increase the rate of particle collision. This causes the microalgal cells in the chamber to migrate toward the nodes of the ultrasonic waves, and to agglomerate. When the irradiation is discontinued, large agglomerates settle rapidly due to gravitation forces. The frequency in the ultrasonic aggregation step is usually on the order of MHz. An appropriate frequency could ensure the adequate distances for particle column separation at appropriate numbers of nodes within acceptable processing time, and meanwhile lowered the possibility of cell disruption or damage due to low acoustic cavitation [209]. However, excess high frequency of the transducer generated acoustic streaming, and disturbed particles stationing at the nodes of standing waves [209]. Excess high frequency also excessively shortened the distances of wave nodes, and possibly made the collection of particles harder.

Bosma et al. [208] reported their work on ultrasound-assisted harvesting of microalgal cells. At optimized conditions (ultrasonic frequency of 2.1 MHz, high biomass concentrations of 3.3×10^8 cells/mL and feeding rate of 5.0 L/day), high efficiencies of ca. 92% and a concentration factor of 11 were possible. The concentration factor is the amount of cells in the harvest flow per the amount of cells in the feeding flow. This research reveals the possibility of ultrasonic energy application on microalgae harvesting on both the laboratory and the pilot scale plant because of its low cost, its compactness, its continuous operation and its lack of fouling characteristics. However, fluid viscosity caused by the concentration of algal cells and the sampling environment such as in fresh or salty water has to be taken into account, because viscous effects may occur for certain frequencies or cell sizes [209].

5.3. *Pretreatment, extraction and conversion of algae*

Ultrasonic irradiation helps in the rupture of algae (microalgae and macroalgae) cells and the reduction of microalgae particle size for better release of chemical contents, and thus improves the extraction efficiency with solvents including supercritical fluids. The enhancement occurs for both the case of microalgae [212–215] and macroalgae [216,217], while the target extractives can be lipids, carbohydrates [75,213,218], proteins [213,219], pigments [220,221] or even adherent contaminants such as polycyclic aromatic hydrocarbons and aliphatic hydrocarbons [216].

Studies on ultrasound-assisted extraction of fatty acids and lipids in microalgae [212,214,215,222–226] and macroalgae [217] have been reported. By soaking the cells in ethyl acetate/methanol mixed solvent and shaking the solution in ultrasound for 3 h, 44.7 wt.% crude lipids were released from *Pavlova sp.* cells, and the extraction efficiency of FAMEs reached 98.1 wt.% [212].

The disruption and extraction of algal cells with high-intensity ultrasound strongly depends on the types of extraction solvent and the species of microalgae. The sequential sonication (about 1.5 W/mL) followed by aqueous mixed enzymes (50 °C, 24–52 h) of *Nannochloropsis* and *Schizochytrium* obtained much lower lipid recovery (16–32% *vs.* 68–95%) than the combination of sonication and ethanol processing (80 °C, 30 min) [227]. For macroalgae, quantitative extraction of total lipids and fatty acids from green (*Ulva fasciata*), red (*Gracilaria corticata*) and brown algae (*Sargassum tenerrimum*) with sonication pretreatment greatly depended on macroalgal species, extraction solvents and buffers. Pre-sonication in $CHCl_3$–CH_3OH mixture (1/2, *v*/*v*, Bligh–Dyer method) or in CH_2Cl_2–CH_3OH mixture (2/1, *v*/*v*, Cequier-Sánchez method) showed remarkable positive effects on extraction efficiency than that with $CHCl_3$–CH_3OH mixture (2/1, *v*/*v*, Folch method) [217]. Sonication at 20 kHz for 16 min caused complete disruption of microalgae *D. salina*, while the cells of microalgae *Nannochloropsis oculata* were barely affected [228].

Algae can be converted into fuels and valuable chemicals. The thermochemical/biochemical conversion of algae materials such as thermal degradation [215] or fermentation for hydrogen [229–231] and biogas [232,233] can be enhanced by additional ultrasonic pretreatment before the reaction. For fermentation of *Chlamydomonas reinhardtii* (a single-celled green algal biomass), the pre-sonication broke down the algal cell walls, released algal starch, and enhanced the production of hydrogen from the starch component [230]. After sonication for 10 min, the degradation ratio of algal starch and H_2 yield reached 85% and 1.8 mol H_2/mol glucose, respectively. Without pre-sonication, the H_2 production rate of intact algae was only 0.2 mL H_2/(L culture h), while it dramatically increased to 47.9 mL H_2/(L culture h) after sonication.

More effective extractions may be realized by combining sonication with other pretreatment methods, such as ball milling [213], enzyme-assisted aqueous extraction [234], microwave irradiation [215] and acid treatment [229]. Combined pretreatment of sonication (20 kHz, 1.5 W/mL) and HCl (concentration, 0.8–1.6%, *v*/*w*) showed much higher efficiency in the dark fermentation of *Chlorella vulgaris* (microalgae) for H_2 production as compared with individual pretreatment [229]. For the pretreatment with ultrasound only, it needed a very high consumption of ultrasonic energy at 80.0 kJ/g DCW (dry cell weight) to reach a H_2 yield of 37.5 mL/g DCW. With the combined pretreatment, high H_2 yield of 42.1 mL/g DCW was achieved with a low ultrasonic energy input of 49.6 kJ/g DCW. The intensification was attributed to easier disruption of the microalgal cell wall structure under sonication after the attack from acid.

Selective conversion of extractives (e.g., polysaccharides, lipids) from macroalgae and microalgae can be further improved through reactions performed directly under ultrasonic irradiation. Zhou and Ma [235,236] studied the degradation of *Porphyra yezoensis* (a red alga) in an ultrasound-assisted circulated extractor. With the increase of ultrasonic power and irradiation time, the molecular weight of polysaccharides in *P. yezoensis* was effectively reduced [235]. The intrinsic viscosity of the solution decreased from nearly 0.34 to 0.08 L/g. The treatment did not destroy the main structure of polysaccharides, while the antioxidant activity of polysaccharides was notably improved [236]. Kinetic calculations demonstrated that due to the intensified mechanical action, the activation energy of *P. yezoensis* degradation under sonication (52.1 kJ/mol) was much lower than that of degradation of polysaccharides with similar glycosidic structure over acid or enzyme catalysts (69–190 kJ/mol) [235].

For biodiesel production from lipid-rich algae, the usual approaches include two-stage processing which means firstly lipid extraction and then transesterification, and one-stage method using direct transesterification of dry materials [237,238]. With direct sonication (20 kHz, 240 W) of the as-harvested *Nannochloropsis* algae for 5 min using CH_3OH–$CHCl_3$ (1:2, *v*/*v*) as solvent and 30 wt.% SrO as catalyst, biodiesel yield of 20.9% of dry biomass was obtained, which was higher than the yield of 7.0% after reflux treatment [237]. The lipid conversion for this case reached 93%. The transesterification of oil from *Oedogonium sp.* (an oil-rich macroalgae) over the immobilized *Bacillus sp.* lipase was intensified directly in an ultrasonic bath at 55 °C [238]. Sonication shortened reaction time significantly from 40 h to only 2 h, with the final FAMEs' yield also improved from 75% to 82%. The increase of methanol/oil molar ratio from 3/1 to 6/1 oppositely decreased the yield from 80.4% to 58.5%. The lipase was thermo-, solvent-, and sono-tolerance, and endured five cycles of sonication, with slight decrease in its catalytic activity.

Sonication shows great potentiality in the extraction of valuable chemicals in macro and microalgae and the conversion of algae to fuels. In the ultrasound-assisted extraction, the type of solvent is of vital importance, as the proper choice of solvent benefits both cavitation intensification and the extraction of algal compositions. However, the disruption of microalgae cells seems to be not an easy work, and consumes too much acoustic energy. The advisable method is to combine sonication with other pretreatments such as mechanical disruption and acid treatment. The conditions for ultrasonic operation also need to be optimized, depending on the species of algae used, while the related researches are still lacking.

5.4. Mechanism and research needs

Enhanced efficiency in lipid or polysaccharide extraction under ultrasonic conditions was mainly ascribed to the disruption of microalgal cells [31,212,231,239]. The photograph taken by energy-filtering SEM (transmission electron microscope) showed that sonication of *Scenedesmus obliquus* YSW15 (microalgae) in bath- or probe-type reactor for 15 and 60 min resulted in remarkable disruption and lysis of algal cells [231]. The nucleus membrane of algal cells was completely lysed, with nucleus materials spreading throughout the cell interior, or even outside the cells. The cell walls were disintegrated, with part of the cells producing vesicle-like features. As time increased, the whole algal cells were completely lysed, and the contents in the cells such as carbohydrates and proteins were excreted to the surroundings or accumulated on the exposed surface of cells and fragments and/or within the periplasm. Therefore, the dissolved fraction of total carbohydrates in the aqueous phase dramatically increased [240]. More algal cell fragments were released to the aqueous phase, with the residual turbidity increased by 24% [240]. The surface roughness of algal cells increased by 4–5 times after sonication, which further increased their superficial area, and possibly benefited the contact of cells with the fermentative bacteria [231]. As a result, the introduction of sonication promoted the accessibility and availability of the carbohydrates in the microalgal cells, and benefits the subsequent fermentation toward ethanol and bio-hydrogen.

However, not all microalgal cells were disrupted in some cases about ultrasound-assisted treatment with different solvents, while the intensified extraction was also observed [31]. When the lipid in *Scenedesmus sp.* cells (microalgae) was extracted with $CHCl_3$–CH_3OH–1% NaCl aqueous solution mixture (3/1/1.2, *v*/*v*, Bligh–Dyer method), only a few microalgal cell clusters was distorted or disrupted, while most other cells remained intact, although their sizes were reduced. However, when the extraction was performed with *n*-hexane and sonicator (20 kHz, about 3.6 W/mL), many clusters were disrupted to a pulpy residue. Nonetheless, intact and shrunken cells were also observed. The lipid extraction rate with the Bligh–Dyer method was higher than that with *n*-hexane and sonicator (2.0% *vs.* 0.8% of dry biomass), while it was even higher with both Bligh–Dyer method and sonicator (6.0%). However, the cavitational intensity in *n*-hexane was instead greater than that in the $CHCl_3$–CH_3OH mixture [31]. The values in *n*-hexane and $CHCl_3$–CH_3OH mixture were 0.65 *vs.* 0.26 cm/s for micro-convection velocity and 23.8 *vs.* 10.0 MPa for high amplitude shockwave, respectively. Therefore, the diffusion of lipid molecules across cell walls was also responsible for the extraction efficiency, which not only resulted from intense cavitation in the studied system, but also depended on the solvents used. Furthermore, this might be the dominant mechanism in the solution with low microbial cell density (e.g., 2 g cells in 20 mL solution) [31].

Additionally, the possible influence of chemical factors such as the generation of free radicals by ultrasonic cavitation is also observed. The formed hydroxyl radical attacks the inner and outer cell surfaces, and greatly enhances the surface hydrophobicity of microalgal cells. The sonication of *S. obliquus* YSW15 with continuous ultrasound in bath at 45 °C for 15 and 60 min significantly decreased the algal surface hydrophobicity from >75% to 54% and 28%, respectively [240]. As heterotrophic microorganisms preferably assimilate hydrophilic organic substrates than hydrophobic ones to a much greater extent, the hydrophilic functional groups on the algal cell surface decreased the aggregation of algae particles, improved the accessibility of substrates to fermenting bacteria and improved the metabolic activity of these bacteria [240]. On the other hand, high-intensity sonication possibly changed the molecular structure of organic matter in algal cells such as arachidonic acid, which was a typical polyunsaturated fatty acid in marine lipid, and resulted in their oxidation and degradation [241]. With sonication at 11.3 W/mL for 10 s, the lipid hydroperoxide value of arachidonic acid was not notably impacted, while with sonication at 4.7 W/mL for 30 s, the hydroperoxide value increased by approximately 30%.

For ultrasonic processing in the disruption and extraction of algae, the selection and optimization of ultrasonic reactors and parameters are very important. Sonication in ultrasonic baths with low power density for short time showed insufficient efficiency in most case of the disruption and extraction of algal cells, with the intensification much less efficient than other pretreatments such as mechanistic shear, thermolysis, microwave and laser treatments [242]. However, excess ultrasonic power and energy input should be avoided. The first reason is to avoid side reactions such as the oxidation of valuable chemicals by active radicals. The second reason is that too much energy input does not mean better performance for algae processing. The release curve of intracellular pigments from the *C. reinhardtii* cells during sonication seemed sharply ascending when the input ultrasonic energy increased from 0 to about 100 J/mL, while it broke and became smooth with further increase of input energy, possibly as the disruption of intact algal cells was nearly complete [241]. Similar phenomenon was also observed by other researchers [240,242].

The following points are recommended and identified as research needs for ultrasound-assisted algae applications:

1) Sonication in the harvesting, extraction and chemical reactions of algae.
2) Harvesting of microalgae under ultrasound standing wave is better in lab- or pilot-plant scale with flow operation than that in industrial scale [208].
3) Seeking for the most energy efficient pretreatment for algal materials by parameter optimization and the combination of ultrasonic energy with other pretreatments such as microwave and acid treatments.
4) Further studies on mechanism analysis. The impact of sonication on cellular morphology, characteristics of the inner and outer cell surfaces, and the release of related chemical compounds should be preferentially studied.
5) The studies on the specificity in different algae species.
6) Simplification of processing steps by *in-situ* thermochemical/biochemical reactions of the as-received algal materials with ultrasonic assistance. However, the adverse influence of impurities such as pigments should be noted.

6. Ultrasonic emulsification and demulsification of crude bio-oil and biomaterials

Mixing and emulsification of crude bio-oil from fast pyrolysis of lignocellulosic biomass with diesel fuel under ultrasonic conditions forms emulsified oil that can be directly used in existing engines [243–246]. By using nonionic emulsifiers (Span-80, Tween-80 and Tween-20) and an additive (*n*-octanol), 10 wt.% of bio-oil aqueous solution was ultrasonically mixed (15–20 kHz, 2–10 W/mL) with 90 wt.% of 0# diesel for 2 h, and yielded a uniform transparent emulsion. The obtained emulsion was stable for nearly 30 days, but it contained high residual carbon after oil vaporization and it had a high viscosity (dynamic viscosity, 5.63–7.37 MPa s at 20 °C *vs.* 3.94 MPa s of 0# diesel) that would need to be reduced for practical applications [243,244].

Another significant topic is the demulsification of oil–water mixtures with the help of ultrasound [247,248]. Contrary to sonoemulsification, ultrasonic demulsification benefits from standing wave effect, and is conducted in similar reactors used in microalgae harvesting with high frequency (400 kHz–2 MHz) and low power (0.02–1 W/mL). Ultrasonic standing waves drive oil droplets (about 2–6 μm) in the dispersion to migrate and cause them to aggregate at the wave nodes. This produces larger droplets or even flocs with sizes greater than 1 mm within a few minutes (Fig. 2c) [247,248]. With ultrasonic irradiation at 420 kHz for 3–5 min and subsequent standing for 30 min, the separation efficiency of canola oil–water emulsion, which was determined as the decrease in visible absorbance at 800 nm, increased from 7% (control group) to 30% (0.02 W/mL for 5 min) and 43% (0.45 W/mL for 3 min) [247]. By combining ultrasonic action and a porous mesh, the separation efficiency could be further improved. Ultrasonic irradiation (680 kHz, 6.3–47.2 W) of soybean oil–water emulsions for about 70 s in a flow reactor equipped with porous polyester–polyurethane reticulated mesh achieved high oil collection rates of 62–80%, compared with less than 10% for collection without ultrasound [248]. It is apparent that the introduction of ultrasonic standing waves can help in separating immiscible products from the reaction system and to recover heterogeneous catalysts more effectively than conventional chemical methods or demulsifiers.

7. Biorefinery process monitoring using low-intensity ultrasound

7.1. Principles of ultrasound monitoring

The *in-situ* or real-time observation, determination and characterization with ultrasound is another avenue of research in biomass pretreatment or reaction system. Ultrasonic detection and measurement systems have attracted much attention as a method for biological and chemical analysis, which is already used in chemical reactions [249,250], interface analysis [13], fluid dynamics and food industry [251]. Ultrasonic measurement has many advantages, such as noninvasiveness and nondestructiveness, lack of necessity for sample preparation, and it can be used in the continuous analysis of opaque, heterogeneous or viscous media over a wide range of temperatures and pressures [252].

In ultrasound measurement, the determination of two basic physical quantities (sound velocity and sound intensity) is of the greatest concern. Many methods can be used for the determination of ultrasonic velocity in real solutions [13]. One common method is to fix the acoustic frequency (such as 1 MHz) and to change the distance between the ultrasonic transmitter and the wave receiver. When the distance becomes an integral multiple of half-wavelength, satisfying the requirement for standing wave formation, the distance is recorded for the calculation of sound velocity. Another method is to determine the resonant frequency for interference generation by changing the ultrasonic frequency in the range of 200 kHz–10 MHz while maintaining the distance between transmitter and receiver. Echo and pulse technologies are introduced for the determination of sound velocity. Considering the relationship of the ultrasonic velocity with the physicochemical properties of the mixed solution, the determination of ultrasonic velocity can provide information on the variation of structure, density, concentration, constitution and phase state of the sample medium.

The determination of acoustic attenuation is an important tool for analyzing solution properties, heterogeneous phase structure and sound absorption of compound molecules. The main factors causing acoustic attenuation can be wave scattering, heat dissipation and molecular absorption. In turbid liquids containing suspended particles, the attenuation of acoustic energy is believed to be related to the scattering of acoustic waves by the small solid particles [13]. Therefore, the concentration, granularity, specific gravity and surface morphology of solid particles in the turbid liquid can be analyzed by determining the decrease in coefficient of the acoustic intensity after ultrasound transmission through the suspension for a certain path length [253,254]. Ultrasonic broadband spectrometry, both for absorption and velocity measurements with scanning frequency from 10 kHz to 10 GHz, has been developed for the purpose of molecular dynamics analysis in physical, chemical and biochemical elementary processes, such as molecular conformational interconversion, mixing/solvation, electrolyte dissociation and structural changes in biomacromolecules [249]. The absorption of ultrasonic energy by different molecules only occurs with some certain ultrasonic frequencies. The energy absorbed would be further transferred to the energy for molecular vibrancy, molecular rotation or certain changes of molecular structure like conformation switchover. It would finally return to the environment through slow relaxation. However, if the ultrasonic frequency is too high so that there is no time to release the energy absorbed back into the environment in an ultrasonic wave cycle, a remarkable attenuation of ultrasonic intensity is observed by ultrasonic broadband spectrometry [255,256].

7.2. Ultrasound measurement in biorefinery

High-frequency ultrasound has been widely used in the determination of properties and composition of various biomass mixtures such as hydrous bio-ethanol [257], mixed fuels containing biodiesel [258,259], oil–water emulsion [260], tetrahydrofuran–methanol/*o*-cresol mixtures [261] and solid sucrose–corn oil suspension [262]. High-frequency ultrasound helps the rapid evaluation of the spoilage of fatty acid composition in plant or waste oil [263]. Various ultrasonic techniques are also used for *in-situ* or real-time analysis of many biomass-related processes, such as the direct real-time monitoring of cellulose saccharification [264,265], the *in-situ* measurement of glycerol settling rate in biodiesel synthesis [266,267] and the real-time analysis of fluidized dynamics in biomass gasifier [268]. Fig. 4 shows several of these applications that are discussed below.

Ultrasound measurement with the frequency of 1–20 MHz allows continuous analysis of enzymatic hydrolysis of disaccharides and microcrystalline cellulose into monosaccharides [252,265] and the fermentation of fruit juices into ethanol [264] (Fig. 4a). The analysis principle is based on the determination of the variation of sound velocity and attenuation. For velocity determination, the experiment uses two identical transducers mounted at the two sides of the reaction container, with the distance of these two sides being fixed (Fig. 4a-1). One transducer is used as emitter for producing ultrasonic pulse with a frequency of 1–4 MHz, while the other is used to receive the signal. The variation of sound velocity propagating in the reacting solution is regarded as the change in mass concentrations of sugar (glucose/fructose) and ethanol in the multicomponent solution. Therefore, by analyzing the time profile of ultrasonic velocity, semi-empirical kinetic models with detailed parameters (e.g., reaction rate profiles, equilibrium constant and molar Gibbs free energy for hydrolysis) could be established in good agreement with the results from discrete assays that were off-line analysis of the concentrations of products and remaining reactants (Fig. 4a-2).

The simultaneous determination of sound velocity, attenuation and other physical quantities like reflection coefficient, backscattering coefficient at more than two frequencies can provide more useful information such as the size reduction of cellulose particles and the formation of bubbles (e.g., CO_2) [264]. With a high-resolution ultrasonic spectroscopy (2–20 MHz), the errors of velocity and attenuation determination could be limited within ±0.0003 m/s and ±0.3%, respectively [265]. Gas bubbles (CO_2) produced in fermentation showed less influential on the ultrasonic velocity. However, a drastic drop in ultrasonic amplitude (attenuation) was observed (Fig. 4a-2) [264].

The reflection of ultrasonic wave is suitable for the analysis of phase interfaces in a heterogeneous system, such as the boundary between glycerol and methyl ester in biodiesel synthesis [266,267]. The experiment apparatus was mainly composed of a measurement cell, an ultrasonic transducer, an ultrasound generator to produce pulse ultrasound with 1.0 MHz broadband central frequency, an oscilloscope and a computer, while the transducer was used as both transmitter and echo receiver (Fig. 4b-1). Based on the acoustic impedance differences between methanol/methoxide phase, glycerol phase and methyl ester phase, part of the ultrasonic wave returns back from the boundary between immiscible phases such as an oil/methoxide interface before mixing or a glycerol/ester interface in reaction, with the rest of the wave being totally reflected from the liquid/air interface (Fig. 4b-2). Then, the boundary location is ascertained by on-line analysis of the pulse–echo ultrasonic waveforms. As the glycerol settles during transesterification, the glycerol/ester interface is heightened, and the time of flight of the ultrasonic pulse in the glycerol phase increases. Thus, the deposition rate of glycerol, which is proportional to the rate of transesterification, can be continuously monitored and determined by the variation of the flight time as a function of reaction time.

Based on the differences in the ultrasonic velocities of chemical components, noninvasive measurement of the distribution of product concentrations, residence time and material mixing in various reactors like tubular or fluidized-bed reactor can be realized using high-frequency (800 kHz) ultrasound technology (Fig. 4c) during the high-temperature chemical reactions such as biomass gasification [268]. The concentration of He in mixed gases can be calculated from the alteration of ultrasonic speed. If solid particles are introduced, a shift in the ultrasonic velocity will occur with additional attenuation (scattering) that decreases the amplitude of the signal. Thus, additional modifications should be made, which means that the calculations are more complicated.

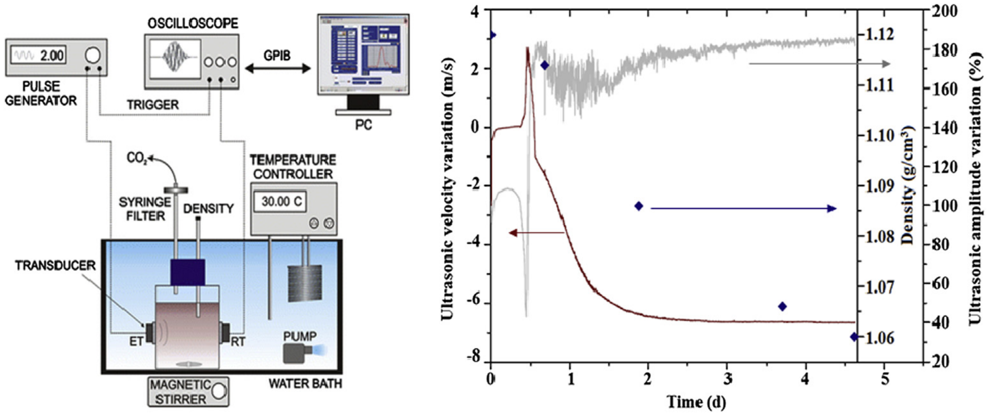
However, it should be noted that there are limitations in the application of ultrasonic measurements [269]. First, only one or two characteristic parameters can be determined for multiphase or multicomponent reactions at a time. Second, the method will be difficult to apply to systems that have high attenuation, such as gas–liquid and gas–liquid–solid reactors with high gaseous concentration (>20%). Therefore, a combination of on-line ultrasound measurement with other methods such as chromatography, spectrometry and mass spectrometry is recommended for such systems.

8. Discussion on critical issues and recommendations

From the discussion above, ultrasonic intensification has already found many applications that can be integrated into a biorefinery, which includes the pretreatment and reactions of lignocelluloses and microalgae, biodiesel synthesis, emulsification/demulsification and process analysis. Furthermore, the potential of ultrasound intensification in other biomass-related pretreatment and reactions, such as lignocellulosic liquefaction to crude bio-oil [92], pre-emulsification of bio-oil for catalytic reforming [270], *in-situ* hydroprocessing of sugars [142,143] and lignin [271], and oxidation of biomass-related compounds such as sugars [272], phenols [273–275] and unsaturated fatty esters [276] are needed.

However, the practical application of ultrasound has some existing barriers:

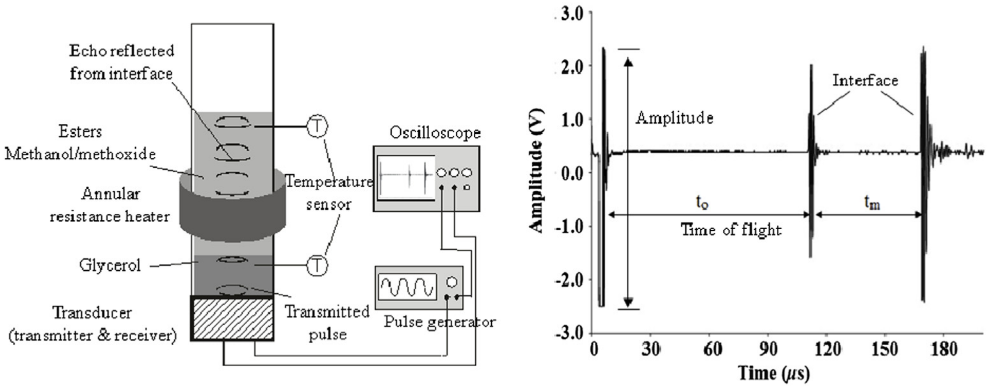
1) Unreasonable ultrasonic processing, such as incorrect choice on sonochemical reactors and acoustic parameters results in poor intensification and unfavorable economics of ultrasonic application. Low intensity of ultrasound and cavitation cannot reach the expected intensification. Excess high intensity brings



(1) Apparatus

(2) Measured curves during wort fermentation

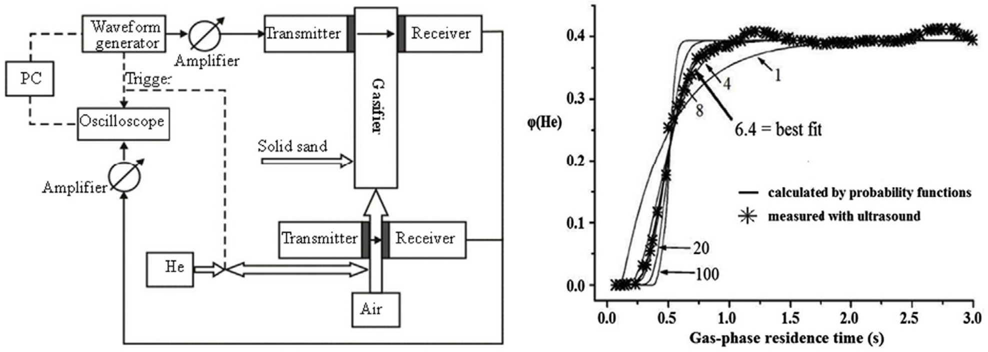
(a) Cellulose saccharification and fermentation



(1) Apparatus

(2) Time variation of ultrasonic parameters

(b) Glycerol settling



(1) Apparatus

(2) Gas-phase residence time distribution curve in the presence of solid particles

(c) Biomass gasification

Fig. 4. Ultrasonic monitoring of biorefinery-related processes. (a: Cellulose saccharification and fermentation. The density of wort is determined by a densitometer, and is proportional to ultrasonic velocity variation). Reprinted with permission from Ref. [264], Copyright ©2009 Springer. b: Glycerol settling. t_o and t_m, time of flight of ultrasound in vegetable oil layer and in methoxide layer, respectively. Reprinted with permission from Ref. [266], Copyright ©2009 Elsevier. c: Biomass gasification. φ(He), volume fraction of He in He–air mixture; numbers in figure c-2 are the numbers of nearly ideal gas-phase mixing reactors with magnetic stirring. Reprinted with permission from Ref. [268], Copyright ©2003 American Chemical Society.)

negative effects on results, and shortens ultrasonic equipment lifetime.

2) The introduction of sonication has low efficiency and is uneconomical in some cases, especially for the scaled equipment and some heterogeneous systems.
3) Development and use of novel heterogeneous chemical catalysts and biological catalysts instead of traditional homogeneous catalysts is attractive. The impact of ultrasonic intensification in the preparation and application of heterogeneous catalysts and enzymes should be studied further. Preparation techniques need experience and theoretical guidance to combine ultrasonic energy with green catalyst synthesis effectively.

These issues will be discussed in the next. The main purpose is to provide some principles for ultrasound applications, and give some classical literature cases and useful recommendations.

8.1. *Selection and optimization of ultrasonic field parameters*

As emphasized in the sections before, the influence of different ultrasonic parameters on the cavitation behavior can be complementary, dependent, and sometimes complicated and conflicting. For instance, cavitational bubbles are difficult to be produced in liquids that are with low vapor pressure or high surface tension, and the population of bubbles decreased, but if the bubbles were formed, high intensity of released energy, as well as strong shockwaves can be expected when they collapse at the positive acoustic pressure phase [40]. Similar puzzles are apparent in the choice of ultrasonic frequency, bulk temperature, additives and aeration conditions, and have been mentioned or discussed in the sections before. A possible strategy is to seek a balance between cavitational density (cavitational yield) and cavitational intensity through the optimization of a set of parameters for certain process using statistical methods such as factorial design and multiple optimization [277]. From the view of energy conversion, the effective ultrasonic intensification should be with three ideal features. First, it should be ascertained that proper cavitational intensity is provided for processing by the sonochemical reactor. Sonication should provide substantial achievements on the pretreatment or reactions of biomass than other methods or that without intensification. This helps us to ensure that the benefit of ultrasonic energy is obtained and the negative influences are minimized. The second is to build up uniform distribution of energy intensity in the whole reactor zone. The third is to obtain similar achievement with less input of ultrasonic energy by the improvement of the yield of effective cavitation. Appropriate balance needs to be made between a more perfect intensification and a higher economic efficiency by avoiding non-essential consumption of ultrasonic energy.

However, the effective conversion of ultrasonic energy is difficult to be realized in the heterogeneous system than in the homogeneous one. It seems necessary to control the concentration of solid reactants and catalysts in the solid–liquid mixture to less than 5–10%, as the impurity phases absorbs, scatters and weakens ultrasonic energy and cavitational intensity [58]. However, for industrial processes, high concentrations of solid reactants are required to avoid the problems regarding process economics and postprocessing. Therefore, the combination of sonication with other pretreatment methods or process intensification technologies is presently the preferred solution. The question is how to achieve better intensification results with creative combinations or integration of multiple methods. On the other hand, the gathering of high density bubble clouds near the sonotrode should be avoided by the multidirection designs of extreme high ultrasonic intensity and timely oscillation of the liquid reactant. For large-scale processing, the uniform distribution of ultrasonic intensity is difficult because of the short-distance action of ultrasound. Therefore, the hydrodynamic characteristics and the distribution of acoustic intensity should be specifically studied, analyzed and theoretically simulated for the entire reactor zone [58]. The advancement on the design of sonochemical reactors is of vital importance.

The selection and optimization of ultrasonic parameters should also consider the mechanism of different chemical reactions and the metabolic pathways of different biological processings. The best choice of parameters such as reaction temperature for ultrasonic operations should benefit both from the lowering of the activation energy and the ultrasonic intensification. On this account, the analysis in the formation, transformation and kinetics of reactive intermediates under ultrasonic conditions may provide useful information, and this could be done through many experimental methodologies such as the trapping or recombination of radicals [29] and the *in-situ* spectroscopy tools.

8.2. *Impact on chemical reactions and heterogeneous catalysis*

For the effective conversion of biomass, catalysis is always one of the most critical factors, which both refers to the catalytic reaction and the catalyst used itself. For the design, preparation, activation and application of catalysts, a catalyst with high activity, high selectivity and the capability of repeatedly reuse is important. For catalytic reactions, the contact of reactants and active sites of catalyst should be sufficient, as well as the detachment of products from active sites in time. For porous catalysts with high surface, the reactant molecules should be able to enter the pores and channels, and to reach the location inside the catalyst that contains the active sites. Many researchers have shown that the introduction of ultrasonic activation plays an important supporting role [30,278].

The introduction of powerful sonication accelerates the hydrolysis, precipitation and crystallization steps in the preparation of heterogeneous catalysts [279–281]. Sonication changes the microscopic morphologies and sizes of solid catalysts, increases the types and amounts of active sites or active phases such as surface acidity/basicity, and benefits the formation of an amorphous or more ordered catalyst structure [281–283]. As a result, catalysts prepared under sonication have improved thermochemical conversion of biomass-related materials, such as the esterification of FFAs over sulfated ZrO_2–TiO_2 solid acid catalyst [280]. For supported catalysts, the introduction of sonication also favors the dispersion, loading or deposition of active components over the supports [281,284,285].

For the catalytic reactions, as mentioned before, ultrasonic cavitation greatly improves the catalyst efficiency. An important indicator to quantitatively assess the improvement of catalyst efficiency is the so-called "turnover frequency" or "turnover efficiency", which is defined as the number of converted reactant molecules over unit number of active sites within unit time. Georgogianni et al. [159] compared the efficiency improvement of homogeneous and heterogeneous catalysts for transesterification reaction of rapeseed oil with methanol under sonication. The turnover frequency over heterogeneous catalysts remarkably increased from 0.001–0.014 to 0.007–0.070 mol/(mol min^{-1}) with ultrasound. But, the turnover frequency over homogeneous NaOH was almost unchanged (0.109–0.211 mol/(mol min^{-1})). The much lower and alterable turnover frequency for heterogeneous catalysts implies the substantial hindrance for the physical/chemical adsorption of reactants over heterogeneous catalysts in the reactions with mechanical stirring, while it is remarkably improved by ultrasonic cavitation [286–288]. In the same way, desorption of the products and by-products from the active sites is accelerated under sonication.

As a result, catalytic kinetics with ultrasound is greatly different from those in conventional reactions, and therefore need further study. The study of the elementary steps in an overall biomass pretreatment scheme or reaction under ultrasonic conditions must be encouraged. The practical mechanism with ultrasound-assisted catalysis should be especially investigated and clarified using proper kinetics models and thermodynamics analysis. As the structure, composition and chemical behavior of raw biomass materials are very complex, reactions occur both on the heterogeneous and homogeneous level and compete against each other, while many secondary reactions such as carbohydrate degradation and lignin monomer condensation seem hard to be eliminated [10]. Therefore, it can be expected that the use of ultrasonic energy can affect competing routes toward target products, by enhancing the mass and heat transfer in critical steps, by improving digestion and dissolution of reactants or by intensifying the formation of radicals, with corresponding improvements in the productivity and selectivity [277]. One of the best cases is the sono-assisted acid hydrolysis of hemicellulose to fermentable sugars [100].

For the reuse of heterogeneous catalysts, the deactivation of catalysts under ultrasonic impact should be carefully evaluated. High-intensity sonication shows both positive and negative effects on the use of heterogeneous catalysts. The negative effects of sonication include the destruction of porous catalyst structure [168] and the loss of active components of the catalyst. However, the loss of active components under ultrasound depends dominantly on ultrasonic intensity, processing time and the catalyst itself. Lewis acid sites over Al_2O_3 might be quickly lost or deactivated under ultrasound-assisted organic reaction [289], while the leaching of active components of some oxides such as CaO and SrO under sonication may be limited as the reaction time is much shortened (Section 4.3).

One of the positive influences of sonication on heterogeneous catalysts is through the timely generation and supplement of new active sites or active phases by cavitational erosion during reactions. This allows catalytic activity to be maintained during biomass hydroprocessing [290]. Sonication also plays a role in continuously removing products and impurities from the catalyst surface, which provides clearer access to active sites over catalysts in conventional catalytic process. A representative case is the *in-situ* regeneration of Raney Ni in the three-phase (solid catalyst–liquid–gas H_2) catalytic hydrogenation of xylose to xylitol (80–130 °C, 4–7 MPa H_2 pressure and 120 min) [142,143]. Ultrasonic irradiation of Raney Ni at 0.2–0.4 W/mL at intervals during hydrogenation reactions maintained the catalyst activity at a constant level (xylitol yield of about 83–89 wt.%). In comparison, the catalyst without midway ultrasonic treatment was deactivated (xylitol yield decreased from 87% to 49%).

As an overall evaluation, ultrasonic energy plays a relative positive influence on the heterogeneous catalysts and catalytic reactions. The intensity of ultrasound should be adapted to different catalysts and reactions by changing the acoustic parameters. Furthermore, advances in the concept and practice of biomass catalysis, as well as in ultrasonic engineering can enhance ultrasonic performance for biomass-related reactions. New catalytic concepts such as phase transfer catalysis [49,291] and emulsion catalysis [292] can be introduced and adopted with ultrasonic irradiation. During the sono-assisted catalytic reaction, emulsion of immiscible liquid reactants can occur so that the heterogeneous catalysts can be well dispersed on the large interface surface of the emulsion [76]. When the reactions are complete, immiscible products [247] and heterogeneous catalysts (Fig. 2d) [293] can be separated or recovered from the residual reactant with the aid of high-frequency ultrasound (>1 MHz).

8.3. Impact on enzymes and biochemical reactions

Biological catalysts such as enzymes have some unique advantages over chemical catalysts for many specific substrates, including high conversion and selectivity, less waste, low pollution, less corrosivity and fewer requirements for the reaction severity, and are therefore regarded as the most promising green processing for biorefinery. The major challenges for enzymatic application are low time efficiency in the cultivation and catalysis reactions, high cost, as well as their ease to be inhibited or inactivated. But, these problems are possibly solved with the assistance of various advanced technologies including sonication. The low-dosage ultrasound was expected to induct and stimulate cultivation and growth of enzymes, and shortened the time needed for the enzymatic cultivation [26]. And moderate ultrasonic intensity actives the enzymes in catalytic reactions, and greatly shortens the time for a reaction cycle. First, ultrasonic cavitation and its secondary effects intensify the adsorption/desorption on the active sites of enzymes, which improves the reaction efficiency and decreases the possible enzymatic deactivation from inhibitors. Second, appropriate high-intensity ultrasound truly influences the morphosis and thus the population and distribution of active sites of enzymes.

Wang et al. [141] revealed how the factors of ultrasound impact the structure and activity of free and immobilized cellulase. Sonication at 18–29 kHz and 0.1–0.8 W/mL for 5–30 min remarkably induced the conformational unfolding or alteration of cellulase proteins. It destroyed the hydrophobic interactions of protein molecules, loosened the cross-linking of immobilized cellulase, and changed the local microstructure of amino acids on cellulases. After sonication at 24 kHz and 0.3 W/mL for 10 min, the structural ratio of α-helix/β-sheet/β-turn/random coil of free cellulase changed from 26.2/26.6/21.9/24.8 to 23.4/25.0/23.7/32.1, and its fluorescence intensity at about 348 nm decreased from nearly 1000 to about 600 a.u. As a result, the activity of free cellulase increased from 50.4 to 59.6 U/mL. However, after sonication at 29 kHz and 0.8 W/mL for 30 min, the fluorescence intensity decreased to less than 500 a.u., while the content of random coil and the activity of free cellulase both decreased to 22.2% and to 31.6 U/mL, respectively. Similar results were reported with lipases (*B. cepacia*) [186]. Far- and near-ultraviolet circular dichroism spectra demonstrated that the tyrosine and tryptophan environments were notably perturbed, and the tertiary structure was also slightly perturbed, accompanied with new formation of smaller irregular spheres with the size of about 0.2–0.5 μm on the surface of this lipase after 1-h sonication in an ultrasonic bath. However, the secondary structure of the lipase was not influenced.

Therefore, it could be concluded that for most enzymatic reactions, sub-lethal doses of ultrasonic irradiation would be beneficial and do not destroy the activities of enzymes [26]. Conformably, another report pointed out that many microorganisms and enzymes seem to be strong enough to endure rigorous manothermosonication, namely combined treatment with ultrasound (about 20 kHz) and heating (60–130 °C) under a certain pressure (0.1–1.0 MPa) [55]. However, care is also to be taken to avoid excessive intensity that can denature the enzyme. Oversonication of *B. cepacia* lipase at longer pre-irradiation time in aqueous buffer showed greatly decreased activity, and therefore made transesterification ratio of ethyl butyrate with butanol worse than that without ultrasonic treatment [186]. The deactivation was mainly from both •OH free radical attack and shear forces, which was the secondary effect of ultrasonic cavitation [61,294].

However, it seemed that ultrasound had synergistic effect with heating on the deactivation of enzymes [61,295]. Ultrasonic stimulation activated α-amylase at hydrolysis temperature of 30–40 °C, while its activity was much lower with ultrasonic irradiation than that in water bath when the temperature increased to over 50 °C [131]. The deactivation of α-amylase was attributed to both thermal denaturation and ultrasonic cavitation [61]. The rate constants for deactivation of α-amylase by thermal denaturation increased with temperature above 50 °C, while that for deactivation caused by ultrasonic effect decreased continuously. As a result, the lowest overall deactivation rate constant appeared at about 50 °C.

It seems difficult to give uniform and accurate values for the enzymatic operation under different conditions such as the determination of acoustic intensity, temperature, time and enzymatic dosage, because different materials and reactions claim different demands to the activities and stability of the enzymes. More importantly, the tolerance of enzyme under special manothermosonication within a giving duration depends on enzyme itself. Different enzymes have different tolerances. Some enzymes such as peroxidase and lipoxygenase are sensitive in the manothermosonication environment and easy to be denatured, while other enzymes such as catalase and yeast invertase were reported to be resistant to ultrasound [294]. For the ultrasound-assisted transesterification, appropriate acoustic power and temperature for *Lipozyme RM IM* were 0.035 W/mL and 40–57 °C, respectively, while they were 0.014 W/mL and 60–70 °C for *Novozym 435* [183]. This hints at the feasibility to seek novel enzyme species that are better adapted to ultrasonic processing [120,296].

Actually, the mechanism of ultrasonic intensification is even different for different reactions, different enzymes and operations [294]. It may be influenced by the high-intensity shearing stress through the vigorous bubble implosion. Or, it depended on the concentration of chemical radicals such as •OH and •H generated by ultrasonic cavitation [297]. The intensification may affect the behavior of enzymes, or only enhance the mass transfer of the reaction system. Therefore, it is better to develop the simulation and characterization of acoustic field features to determine the main intensification causes, and to help building a more suitable system for the treatment and reactions of enzymes. The selection of ultrasonic parameters is not unalterable, and any optimization depends on the specific treatments. For instance, very high-frequency order of MHz may result in the maximum increase (200%) in the activity of immobilized α-amylase and glucoamylase [26].

The characteristics of solvents and water content also affect the activities and thermostabilities of enzymes. The properties of mixed solvent systems, such as partition coefficients, dielectric constants and solubility parameters, have a strong influence on enzyme activity under ultrasound. Mixed solvents containing branched alcohols (*tert*-amyl alcohol and *tert*-butanol) as cosolvent show the best performance among various solvents including hexane, pentanol, cyclohexanol, butanol, acetone and 1 4-dioxane with linear or branched structure [182]. When water content was 0.5%, *Novozym 435* exhibited its highest activity of about 670 μmol/(min g) under sonication in 2 h [182]. However, it sharply declined to 260 μmol/(min g) for a water content of 4%. Other reports also showed that the enzyme structure was stable in nearly anhydrous organic solvents [180,186]. With water content of 0.5%, the activity of immobilized enzymes (*Candida antarctica lipase B*) that was recovered after transesterification had only a slight decrease of 4% after five reaction cycles [182]. This might be attributed to two factors. First, some unexpected changes of enzyme structure may occur in enzymatic catalysis in aqueous solutions, which is related to the enzyme activity and thermostability [186,298]. Second, contrary to conventional thinking, many enzymes do not lose their native structure and catalytic activity in nearly pure organic solvents, because the rigid enzyme molecules are not flexible in the absence of water [299]. The unbeneficial influence of excess water happens for all reactions containing lipase in aqueous systems, while it might be amplified under ultrasonic irradiation when considering the enhanced attacking of •OH by cavitation. However, the detrimental effects of water content might be carefully evaluated with various enzymes, considering the specificity of different enzymes.

Another feasible method to improve the stability of enzymes is the immobilization of free enzymes. Immobilization may loss some activity of free enzymes, however, it benefits the recovery and reuse of enzymes. Compared with free cellulase, immobilized cellulase can endure longer duration of sonication with higher acoustic intensity [141]. Considering the limitation of mass transfer in heterogeneous system in the case of immobilized enzymes, it was speculated that sonication may give greater benefits to the immobilized enzymes. However, in actual enzymatic reactions, sonication is not partial to free or immobilized enzymes. The increase of surface area of immobilized cellulase after ultrasonic treatment resulted in slight higher enhancement in enzymatic activity of immobilized cellulase than that of free cellulase [141]. However, in the ultrasound-assisted glycerol conversion over free or immobilized *C. pasteurianum*, the contribution of ultrasound on the improvement of 1,3-propanediol yield over immobilized enzymes is less pronounced than that over free enzymes [194]. The reason is attributed to the fact that ultrasound can get rid of the great substrate inhibition over free enzymes, while the substrate inhibition is partly solved in the case of immobilized enzymes [194].

As recommendations, the following points are proposed for treating the biomass reaction system with enzymes:

1) Integrated optimization of parameters for enzymatic operation. The critical parameters include acoustic parameters, temperature and enzymatic concentration. The acoustic parameters include ultrasonic intensity in the actual reactor, sonication model (pulse or continuous), acoustic frequency, acoustic impedance and others. Other factors such as static pressure, treating time, aerating operation and solvent characteristics should also be considered.
2) Optimization of ultrasonic operation for different enzymes based on the analysis of the real intensification mechanism.
3) Understanding of the relationship of the variance between enzymatic structure and ultrasonic irradiation, the variance of the population and distribution of active sites with the changed apparent kinetics of enzymatic reactions. The variance of apparent morphology such as knotting, uniting and disintegration of enzymatic communities can be observed by electron microscopy technologies. Circular dichroism method, nuclear magnetic resonance analysis and spectroscopy methods such as ultraviolet difference and fluorescence could be used to identify the macromolecular conformation of enzymes, with the amino acid composition and microenvironment being checked. The variances of intra and intermolecular interactions of the enzyme molecules could be studied with advanced technologies such as atomic force microscopy. These will help in determining the most appropriate surroundings for the use of enzymes.
4) Cultivation and recovery through studies on ultrasound-assisted cultivation and population increase of enzymes, as well as their release and recovery from biological cells [42].

8.4. Energy and processing benefit assessment in case studies

The process economics or energy balance of ultrasonic application should be carefully evaluated for certain pretreatment or reactions. Typical cases with the production of biofuels from

Table 4
Energy and economic trade-off assessments.

Ultrasound-assisted processes	Materials	Ultrasonic parameters[a]	Energy efficiency analysis	Assessment	References
Pretreatment for protein and sugar extraction	Defatted soy flakes	Probe, 20 kHz, 0.30–2.56 W/mL, 15–120 s, 100 g defatted soy flakes in 500 mL solution	Protein yields improved by 8% and 25% and sugar yields improved by 2.5 and 4.6 g per 100 g defatted soy flakes, while an additional energy input of 36 and 307 J/mL is required.	Negative	[85]
Pretreatment for starch hydrolysis	Sugary-2 maize	Probe, 20 kHz, 4.8–8.3 W/mL, 5–40 s, Concentration of 3 g/32 mL	With medium (6.3 W/mL) and high power density (8.3 W/mL), Energy(output)/Energy(input) = 2.0–5.0 kJ/kJ.	Positive	[116]
Pretreatment for the saccharification and fermentation to ethanol[b]	Cassava chip	Probe, 20 kHz, 8.5 W/mL, Sonication time of 10–30 s, Slurries with 5% total solids (TS)	1) Energy consumption and ethanol yield of sonicated sample were 3.2 kJ/g TS and 43.05 wt.%, close to control group (0 kJ/g TS, 31.3 wt.%) but shortened fermentation time from 70 h to 20 h. 2) Sonication was superior to cooking. For cooked sample, energy consumption and ethanol yield were 6.3 kJ/g TS and 33.5 wt.%.	Positive	[118]
Fermentation to ethanol	Glucose	Bath, 40 kHz, about 0.027 W/mL, 20 or 30 °C, 4–20 h, Glucose (20–40 wt.%), *Saccharomyces cerevisiae*	With additional sonication energy of 0.17×10^{-3} kJ, 10 g glucose (heating value of 160.3 kJ) was totally converted to ethanol (heating value of 143.9 kJ), with reaction time shortened from 18 h to 11 h.	Positive	[129]
Pretreatment of cellulose before saccharification[c]	Microcrystalline cellulose	Probe, 20 kHz, 1.5 W/mL, 80 °C, 3 h, Solid/liquid ratio of 5 g/200 mL	With additional sonication energy of 648 kJ/g cellulose, increase of glucose yield gave additional energy output of 1.68–2.64 kJ/g cellulose.	Negative	[87]
Enzymatic hydrolysis of cellulose[c]	Newspaper, office paper, pulp	Probe, 0.8–6.4 L reactor, 20 kHz, 30 or 60 W/(calorimetry), 45 °C, 48 h, Substrate concentration of 7.5–25 g/L, Cellulase (from *Trichoderma viride*)	With additional sonication energy of 5.2–10.4 MJ, increased total sugar yield gave additional energy output of 15–160 kJ.	Negative	[78]
Homogeneous base-catalyzed biodiesel synthesis	Soybean oil	Pilot reverberative flow reactor, 45 °C, 55 mL/min, Methanol /oil/30% CH$_3$COONa, mass ratio of 80/19.5/0.5, First mechanical stirring for 30 min, Then sonication (21.5 kHz, 0.12 W/mL) for 35 min	Energy consumption was about 1.01 MJ/L biodiesel. Low heating value of soybean biodiesel is about 33 MJ/L [5].	Positive	[54]
		Probe, continuous/pulse, 20 kHz, 3.5–8.7 W/mL, 48–60 °C, 60–90 s (sonication for 15–20 s), Methanol/oil molar ratio of 3/1 NaOH (0.1 mg/10 mL oil)	For pulse sonication, continuous sonication and commercial stirring, energy consumptions are 110, 112 and 162 kJ/L reaction mixture, highest biodiesel yields are 96%, 86% and 97%, and reaction times are 2 min, 15 s and 60 min.	Positive	[153]
		Probe ultrasonic, 611 kHz, 0.31 W/mL, 26 °C, 25 min, Methanol/oil molar ratio of 6/1, 0.5% KOH.	Energy consumption with ultrasound is 0.27 kJ/g biodiesel. Low heating value of soybean biodiesel is about 37.2–37.9 kJ/g [5]. Biodiesel yield increases from 45% with magnetical stirring (1.89 W/mL, 45 min) to 82% with sonication (0.31 W/mL, 25 min).	Positive	[165]
	Sunflower oil	Flat plate or probe, 28 kHz, Estimated 2–3 W/mL 50 °C, 30–50 min Methanol/oil molar ratio of 5/1–10/1, NaOH, 1.0–1.5 wt.%	Comparison of four intensification methods: mechanical stirring (MS): flat plate sonication (FPUI); flat plate sonication + stirring (UIMS); probe sonication (PUI). Energy consumption: PUI (0.648 MJ) < UIMS (0.684 MJ) < FPUI (0.864 MJ) < MS (1.116 MJ). Biodiesel conversion of 95%.	Positive	[155]
	Pre-esterified *Nagchampa* oil	Probe, 20 kHz, estimated rating power of 1 W/mL, 50 or 88 °C, 6–20 min	Comparison of four intensification methods: conventional reflux (CR): probe sonication (US); microwave radiation (MW); microwave + sonication (MW/US). Energy consumption: MW/US (0.07 MJ) < MW (0.25 MJ) < US (0.56 MJ) < CR (1.24 MJ). Biodiesel conversion of 89–91%.	Positive	[79]

(*continued on next page*)

Table 4 (*continued*)

Ultrasound-assisted processes	Materials	Ultrasonic parameters[a]	Energy efficiency analysis	Assessment	References
Release of total sugars for fermentation[c]	Microalgae *Scenedesmus obliquus* YSW15	Bath, 40 kHz, about 0.043 W/mL, 45 or 65 °C, 15 min, Microalga concentration of 34 mg/mL	With sonication at 0.043 W/mL for 15 min, utilization of total sugars improved from 0.15 to 0.29 g/g biomass.	Positive	[240]
Pretreatment for fermentation to hydrogen production[b]	Microalgae *Chlorella vulgaris*	Probe, 20 kHz, 1.5 W/mL, 78 °C, 10–120 min. Liquid/solid mass ratio of 10/1	1) Acid pretreatment, 1.6 wt.% HCl, H_2 yield of 36.5 mL/g DCW (dry cell weight); 2) Sonication, specific energy input of 80 kJ/g DCW, H_2 yield of 37.5 mL/g DCW; 3) Acid + sonication, 1.6 wt.% HCl, specific energy input of 55 kJ/g DCW, H_2 yield of 41.5 mL/g DCW.	Negative	[229]
Pretreatment for fermentation to methane production[b]	Microalgae *Scenedesmus*	Probe, 20 kHz, 2.1 W/mL, Specific supplied energy of 128.9 kJ/g, 85 °C, 30 min.	Methane yield of pre-sonicated sample improved from 128.7 (preheated at 80 °C for 25 min) to 153.5 mL CH_4/g COD after 34-day fermentation, with higher energy requirements for pre-sonication.	Negative	[232]
	Mixed microalgae (*Chlamydomonas*, *Scenedesmus*, *Nannochloropsis*, *Acutodesmus obliquus*, *Oocystis sp.*, *Phormidium Nitzschia sp*, *Microspora*)	Probe, 20–25 kHz, maximum rating power density of 2 W/mL, About 25–285 s Microalgae concentrations of 9–10 g TS (total solid)/kg	Methane yield of pre-sonicated sample improved by 20.8–53.1 mL/g TS, providing additional combustion heat of 0.8–2.1 kJ/g TS, while additional ultrasonic energy of 10–57 kJ/g total solid was required.	Negative	[233]

[a] Power levels additionally marked with word "calorimetry" are actually determined using calorimetry method, while others without labels are the power rating of the device.

[b] Heats of combustion of ethanol, hydrogen and methane are 1366.8, 285.8 and 890.8 kJ/mol, respectively [72].

[c] Heating value of glucose is 2888 kJ/mol [129].

lignocellulosic biomass, lipids and algae resource are compared in Table 4, and summarized below:

1) The net energy yield and processing benefit seems positive for ultrasound-assisted biodiesel synthesis.
2) The energy economics are positive for the ultrasound-assisted conversion of glucose and starch. The sonication at 24 and 500 kHz in the acid hydrolysis of potato starch had better performance and energy economics than mechanical stirring and microwave assistance [119].
3) With current sonication technologies, additional sonication in the pretreatment or thermochemical/biochemical reactions of cellulosic biomass to sugars and alcohols consumes much greater ultrasonic energy, while the increased energy output as the result of increased sugar yields is only several percentages of the consumed energy. High-intensity ultrasound partly destroys the crystalline state of cellulose, and thus has notable effect on the improvement of cellulose accessibility, while it has less or even negative influence on lignin degradation [300]. In the beginning of ultrasound-assisted direct enzymatic saccharification, the improvement of enzymatic efficiency may be not remarkable possibly because of the existence of recalcitrant lignocellulosic structures [56].
4) Ultrasonic extraction of proteins and sugars from defatted soy flakes seems uneconomical [85]. Although the total release of proteins and sugars remarkably increased with sonication, higher additional consumption of ultrasonic energy up to 92–154 kJ per 100 g of defatted soy flakes was provided.
5) The economic assessments on the ultrasonic extraction of lipids [301] from microalgal cells and the sono-assisted chemical/biochemical reactions of algae [215,232,233] are not encouraging, and are possibly poorer than other pretreatments such as microwave and laser treatments [242].

The low efficiency and uneconomics of biofuel production with high-intensity ultrasound could be mainly due to the following factors:

1) The purpose for ultrasound application is with high ultrasonic energy consumption, while no other methods were combined or integrated in the ultrasonic operation. This problem is highlighted in the case of cellulose treatment. For example, the particle size of sawdust slurries was reduced to less than 1 μm under high-intensity ultrasound [84]. This surely needed very high-energy consumption of ultrasound, while it did not result in prominent improvement in the yield and rate in enzymatic saccharification. Similar situations are possible for the degradation of the crystallinity degree of cellulose, the formation of very fine emulsion in viscous liquid and other energy intensive processings.
2) The choice of the materials for ultrasonic operation, as well as the economics of some target products, is questionable. The sonication of full-fat soybean flakes after extrusion process also showed unremarkable intensification on enzymatic saccharification, possibly because the cellular structure had already been completely disrupted by the former pretreatment [302]. Ultrasonic pretreatment of cellulose for the enhancement of subsequent subcritical liquefaction obtained much negative energy yield, which is due to the poor fuel properties of the obtained bio-oils [92].
3) The selected sonochemical reactor is not appropriate for ultrasonic operation in the specific reactions. The distribution of ultrasonic energy in the scaled reactor was seriously nonuniform, as discussed before.
4) The selected parameters and operations do not meet the energy requirement for the specific reactions, as discussed before.

5) For the treatment and conversion of microalgae, the low process economics may be attributed to the low mass concentration of 1–10 wt.% of algae suspension and the relative low extractable or degradable content of lipids (<50–60%) or polysaccharides (40–50%) in the algal materials. Single-celled morphology of microalgae is not easy to be degraded by ultrasound, and may require for ultrasonic intensity higher than that in starch treatment and biodiesel synthesis with other lipid materials.
6) Micron-/nano-size of lignocellulosic particles in suspension, as well as high volume density of small microalgal cell clusters in the liquid reactant results in serious attenuation of ultrasonic energy.

In consequence, the suggestions are made for the improvement of process economics:

1) To combine or integrate the ultrasonic treatment with other intensification methods, especially for processing high solid concentration of biomass feedstock in large-scale.
2) To intensify timely separation of products from the reacting systems.
3) To optimize the reactor design and operating parameters.
4) To carry on the studies about the kinetics and mechanism in ultrasound-assisted reactions. The required activation energy levels for different reactions should be analyzed to find the best combination of ultrasonic intensification with chemical activation.
5) To optimize the reaction course by process monitoring (including that with low-intensity ultrasound).
6) To intensify the preparation and reactions of the heterogeneous catalysts and enzymes by ultrasonic energy. This includes the reduction of the cost in the catalyst preparation, the improvement of the properties and performance of the catalysts, the improvement of the recycling and reuse of the catalysts, and the prolonging of the catalyst lifetime.
7) To increase the process economics by the optimized raw materials and the utilization of the process by-products. The added economic values of the by-products such as glycerol and the waste extracts should be promoted, possibly with ultrasound technologies.
8) The selection of algal species, solvent-free extraction [225] and the combination with other pretreatments [229] are suggested for improving the process economics with algal resource.

9. Conclusions

Ultrasonic energy has a special place in meeting the challenges of processing recalcitrant, multicomponent and heterogeneous biomass materials. The introduction of an ultrasonic field can provide an extremely severe physicochemical environment that is difficult to realize with other engineering methods. Sonication does not remarkably change the chemical mechanism of biomass pretreatment and reactions, but the reaction kinetics is remarkably accelerated as the result of ultrasonic cavitation and the secondary effects, and therefore enhances the efficiency and economics of the biomass conversion process. It can be concluded that:

1) For the conversion of lignocellulosic biomass, the combination of ultrasonic energy and proper solvents allows the destruction of the recalcitrant lignocellulosic structure, facilitates the solvation and fractionation of biomass components, and finally benefits the acceleration of catalytic conversion rate and the increase of equilibrium yields of sugars, ethanol, bio-hydrogen and others products. This is attributed to the reduction of solid biomass size, the increase of cellulose hydrophilicity, the improvement of mass transfer in reactions and the activation of catalysts.
2) The ultrasonic cavitation generates violent shockwaves and micro-convection that can break the immiscible binary reactants – oils and alcohols into tiny emulsion, and therefore enhance the hydrolysis, esterification and transesterification reactions in biodiesel synthesis. The ultrasonic intensification do not change the chemical mechanism of transesterification and other reactions, however, good results for these reactions will be benefited from both chemical activation and cavitational intensification.
3) Sonication favors the cultivation, harvesting, component extraction and conversion of macro and microalgae to sugars, ethanol, bio-hydrogen and biodiesel. The main mechanisms should be attributed to the effective disruption and thus the lysis of algal cells under high-intensity sonication. The intensified diffusion of the extractive molecules such as lipids across cell walls, as well as the improved hydrophily of the algal cell surface, also contributes the pretreatment performance. As a result, the accessibility and availability of the algal cells is greatly improved.
4) Ultrasound with high frequency of MHz orders benefits the demulsification of the immiscible emulsion, the recovery of solid particles and the *in-situ* monitoring of biorefinery process. These technologies may be useful for the product separation, catalyst recovery and the determination of the best reaction conditions in the future researches on the treatment and conversion of biomass-related materials.
5) High-intensity ultrasound helps for the preparation and activation of heterogeneous catalysts. The catalytic kinetics may be changed through the enhanced adsorption and desorption over the catalyst surface, and therefore benefits the catalytic efficiency and the reuse and lifetime of the catalysts.
6) For biochemical reactions, ultrasound stimulation changes the microenvironments in the chemical structure of the enzyme molecules, and thus improves the activity for enzymatic reactions. However, the combined conditions for heating and sonication should be carefully controlled to avoid unnecessary loss of the enzyme activities. The optimization of the operation parameters should be carefully performed on different enzymes, as the tolerance of different enzyme within the same sonication and heating cycles may have a great difference.

As a conclusion, the introduction of ultrasonic energy plays a positive influence on the pretreatment and thermochemical/biochemical conversion of biomass. The current barriers that hinder the large-scale application of ultrasonic energy could be those at the scientific level (such as the real mechanism of ultrasonic intensification and its integration with chemical energies), those at the engineering level (such as the improvement of ultrasonic reactor design and parameter optimization) and those at the economic level (such as the improvement of energy efficiency and lowering the cost for ultrasonic operation and the catalysts). Therefore, ultrasound-assisted processes are needed to be further studied both at the fundamental level and at the applicable level to realize practical ultrasonic systems.

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References

[1] U.S. Energy Information Administration. Annual energy outlook 2011 with projections to 2035. Washington DC: U.S. Energy Information Administration; 2011 April. Report No.: DOE/EIA-0383.

[2] Kamm B, Gruber PR, Kamm M. Biorefineries-industrial processes and products: status quo and future directions. 1st ed. Weinheim: Wiley-VCH; 2006.

[3] Huber GW, Iborra S, Corma A. Synthesis of transportation fuels from biomass: chemistry, catalysts, and engineering. Chem Rev 2006;106:4044–98.

[4] Marchetti JM, Fang Z. Biodiesel: blends, properties and applications. New York: Nova Science Publishers; 2011.

[5] Fukuda H, Kondo A, Noda H. Biodiesel fuel production by transesterification of oils. J Biosci Bioeng 2001;92:405–16.

[6] Brennan L, Owende P. Biofuels from microalgae – a review of technologies for production, processing, and extractions of biofuels and co-products. Renew Sustain Energy Rev 2010;14:557–77.

[7] Ferrell J, Sarisky-Reed V. National algal biofuels technology roadmap. Washington, DC: US Department of Energy, Office of Energy Efficiency and Renewable Energy, Biomass Program; 2010 May. Report No.: DOE/EE-0332.

[8] Chundawat SPS, Bellesia G, Uppugundla N, da Costa Sousa L, Gao D, Cheh AM, et al. Restructuring the crystalline cellulose hydrogen bond network enhances its depolymerization rate. J Am Chem Soc 2011;133:11163–74.

[9] Yu Y, Lou X, Wu HW. Some recent advances in hydrolysis of biomass in hot-compressed water and its comparisons with other hydrolysis methods. Energy Fuel 2008;22:46–60.

[10] Huber GW. Breaking the chemical and engineering barriers to lignocellulosic biofuels: next generation hydrocarbon biorefineries. Washington, DC: National Science Foundation; 2008 March.

[11] Mettler MS, Vlachos DG, Dauenhauer PJ. Top ten fundamental challenges of biomass pyrolysis for biofuels. Energy Environ Sci 2012;5:7797–809.

[12] Lifka J, Ondruschka B. Influence of mass transfer on the production of biodiesel. Chem Eng Technol 2004;27:1156–9.

[13] Ensminger D, Bond LJ. Ultrasonics: fundamentals, technologies, and applications. 3rd ed. Boca Raton: CRC Press; 2011.

[14] Iskalieva A, Yimmou BM, Gogate PR, Horvath M, Horvath PG, Csoka L. Cavitation assisted delignification of wheat straw: a review. Ultrason Sonochem 2012;19:984–93.

[15] Bussemaker MJ, Zhang DK. Effect of ultrasound on lignocellulosic biomass as a pretreatment for biorefinery and biofuel applications. Ind Eng Chem Res 2013;52:3563–80.

[16] Shirsath SR, Sonawane SH, Gogate PR. Intensification of extraction of natural products using ultrasonic irradiations – a review of current status. Chem Eng Process 2012;53:10–23.

[17] Kardos N, Luche JL. Sonochemistry of carbohydrate compounds. Carbohydr Res 2001;332:115–31.

[18] Veljković VB, Avramović JM, Stamenković OS. Biodiesel production by ultrasound-assisted transesterification: state of the art and the perspectives. Renew Sustain Energy Rev 2012;16:1193–209.

[19] Badday AS, Abdullah AZ, Lee KT, Khayoon MS. Intensification of biodiesel production *via* ultrasonic-assisted process: a critical review on fundamentals and recent development. Renew Sustain Energy Rev 2012;16:4574–87.

[20] Gole VL, Gogate PR. A review on intensification of synthesis of biodiesel from sustainable feed stock using sonochemical reactors. Chem Eng Process 2012;53:1–9.

[21] Bhaskaracharya RK, Kentish S, Ashokkumar M. Selected applications of ultrasonics in food processing. Food Eng Rev 2009;1:31–49.

[22] Pilli S, Bhunia P, Yan S, LeBlanc RJ, Tyagi RD, Surampalli RY. Ultrasonic pretreatment of sludge: a review. Ultrason Sonochem 2011;18:1–18.

[23] Yin X, Han PF, Lu XP, Wang YR. A review on the dewaterability of bio-sludge and ultrasound pretreatment. Ultrason Sonochem 2004;11:337–48.

[24] Gogate PR, Kabadi AM. A review of applications of cavitation in biochemical engineering/biotechnology. Biochem Eng J 2009;44:60–72.

[25] Rokhina EV, Lens P, Virkutyte J. Low-frequency ultrasound in biotechnology: state of the art. Trends Biotechnol 2009;27:298–306.

[26] Kwiatkowska B, Bennett J, Akunna J, Walker GM, Bremner DH. Stimulation of bioprocesses by ultrasound. Biotechnol Adv 2011;29:768–80.

[27] Suslick KS. Sonochemistry. Science 1990;247:1439–45.

[28] Shah YT, Pandit AB, Moholkar VS. Cavitation reaction engineering. New York: Kluwer Academic/Plenum Press; 1999.

[29] Ashokkumar M. The characterization of acoustic cavitation bubbles – an overview. Ultrason Sonochem 2011;18:864–72.

[30] Suslick KS, Skrabalak SE. Sonocatalysis. In: Ertl G, Knözinger H, Schüth F, Weitkamp J, editors. Handbook of heterogeneous catalysis. 2nd ed. Weinheim: Wiley-VCH Verlag GmbH& Co. KGaA; 2008. pp. 2007–17.

[31] Ranjan A, Patil C, Moholkar VS. Mechanistic assessment of microalgal lipid extraction. Ind Eng Chem Res 2010;49:2979–85.

[32] Moholkar VS, Warmoeskerken MMCG. Integrated approach to optimization of an ultrasonic processor. AIChE J 2003;49:2918–32.

[33] Sutkar VS, Gogate PR. Design aspects of sonochemical reactors: techniques for understanding cavitational activity distribution and effect of operating parameters. Chem Eng J 2009;155:26–36.

[34] Sutkar VS, Gogate PR, Csoka L. Theoretical prediction of cavitational activity distribution in sonochemical reactors. Chem Eng J 2010;158:290–5.

[35] Moholkar VS, Sable SP, Pandit AB. Mapping the cavitation intensity in an ultrasonic bath using the acoustic emission. AIChE J 2000;46:684–94.

[36] Sutkar VS, Gogate PR. Mapping of cavitational activity in high frequency sonochemical reactor. Chem Eng J 2010;158:296–304.

[37] Gogate PR, Tatake PA, Kanthale PM, Pandit AB. Mapping of sonochemical reactors: review, analysis, and experimental verification. AIChE J 2002;48:1542–60.

[38] Tsochatzidis NA, Guiraud P, Wilhelm AM, Delmas H. Determination of velocity, size and concentration of ultrasonic cavitation bubbles by the phase-Doppler technique. Chem Eng Sci 2001;56:1831–40.

[39] Gogate PR, Tayal RK, Pandit AB. Cavitation: a technology on the horizon. Curr Sci 2006;91:35–46.

[40] Thompson LH, Doraiswamy LK. Sonochemistry: science and engineering. Ind Eng Chem Res 1999;38:1215–49.

[41] Mason TJ, Paniwnyk L, Lorimer JP. The uses of ultrasound in food technology. Ultrason Sonochem 1996;3:S253–60.

[42] Gogate PR, Pandit AB. Application of cavitational reactors for cell disruption for recovery of intracellular enzymes. J Chem Technol Biotechnol 2008;83:1083–93.

[43] Zhang CC, Lu XP, Han PF. Application of liquid whistle ultrasonic generator for circulation water sterilization. Chem Reaction Eng Technol 2012;28:70–86 [in Chinese].

[44] Singiser RE, Beal HM. Emulsification with ultrasonic waves II. Evaluation of three ultrasonic generators and a colloid mill. J Am Pharm Assoc 1960;49:482–7.

[45] Shi GB, Shi Y, inventor; Shanghai Shangao Energy Saving Sci & Technology Co Ltd, assignee. Fluidic power ultrasonic emulsifying device useful for heavy oil comprises oil inlet, oil filter, high pressure pump, water tank and filter, oil and water heating device, pre-mixer, and first and second reed whistle ultrasonic emulsifier. Chinese patent: CN201197931-Y; 25 Feb 2009 [in Chinese].

[46] Davidson RS, Safdar A, Spencer JD, Robinson B. Applications of ultrasound to organic chemistry. Ultrasonics 1987;25:35–9.

[47] Chakinala AG, Gogate PR, Burgess AE, Bremner DH. Treatment of industrial wastewater effluents using hydrodynamic cavitation and the advanced Fenton process. Ultrason Sonochem 2008;15:49–54.

[48] Faïd F, Contamine F, Wilhelm AM, Delmas H. Comparison of ultrasound effects in different reactors at 20 kHz. Ultrason Sonochem 1998;5:119–24.

[49] Entezari MH, Keshavarzi A. Phase-transfer catalysis and ultrasonic waves II: Saponification of vegetable oil. Ultrason Sonochem 2001;8:213–6.

[50] de La Rochebrochard S, Suptil J, Blais JF, Naffrechoux E. Sonochemical efficiency dependence on liquid height and frequency in an improved sonochemical reactor. Ultrason Sonochem 2012;19:280–5.

[51] Faïd F, Romdhane M, Gourdon C, Wilhelm AM, Delmas H. A comparative study of local sensors of power ultrasound effects: electrochemical, thermoelectrical and chemical probes. Ultrason Sonochem 1998;5:63–8.

[52] Entezari MH, Pétrier C, Devidal P. Sonochemical degradation of phenol in water: a comparison of classical equipment with a new cylindrical reactor. Ultrason Sonochem 2003;10:103–8.

[53] Loranger E, Paquin M, Daneault C, Chabot B. Comparative study of sonochemical effects in an ultrasonic bath and in a large-scale flow-through sonoreactor. Chem Eng J 2011;178:359–65.

[54] Cintas P, Mantegna S, Gaudino EC, Cravotto G. A new pilot flow reactor for high-intensity ultrasound irradiation. Application to the synthesis of biodiesel. Ultrason Sonochem 2010;17:985–9.

[55] Yachmenev V, Condon B, Klasson T, Lambert A. Acceleration of the enzymatic hydrolysis of corn stover and sugar cane bagasse celluloses by low intensity uniform ultrasound. J Biobased Mater Bioenergy 2009;3:25–31.

[56] Easson MW, Condon B, Dien BS, Iten L, Slopek R, Yoshioka-Tarver M, et al. The application of ultrasound in the enzymatic hydrolysis of switchgrass. Appl Biochem Biotechnol 2011;165:1322–31.

[57] Cucheval A, Chow RCY. A study on the emulsification of oil by power ultrasound. Ultrason Sonochem 2008;15:916–20.

[58] Gogate PR, Sutkar VS, Pandit AB. Sonochemical reactors: important design and scale up considerations with a special emphasis on heterogeneous systems. Chem Eng J 2011;166:1066–82.

[59] Asakura Y, Nishida T, Matsuoka T, Koda S. Effects of ultrasonic frequency and liquid height on sonochemical efficiency of large-scale sonochemical reactors. Ultrason Sonochem 2008;15:244–50.

[60] Kumar A, Gogate PR, Pandit AB. Mapping the efficacy of new designs for large scale sonochemical reactors. Ultrason Sonochem 2007;14:538–44.

[61] Kadkhodaee R, Povey MJW. Ultrasonic inactivation of *Bacillus* α-amylase. I. Effect of gas content and emitting face of probe. Ultrason Sonochem 2008;15:133–42.

[62] Vichare NP, Senthilkumar P, Moholkar VS, Gogate PR, Pandit AB. Energy analysis in acoustic cavitation. Ind Eng Chem Res 2000;39:1480–6.

[63] Seymour JD, Wallace HC, Gupta RB. Sonochemical reactions at 640 kHz using an efficient reactor. Oxidation of potassium iodide. Ultrason Sonochem 1997;4:289–93.

[64] Hua I, Hoffmann MR. Optimization of ultrasonic irradiation as an advanced oxidation technology. Environ Sci Technol 1997;31:2237–43.

[65] Crum LA. Comments on the evolving field of sonochemistry by a cavitation physicist. Ultrason Sonochem 1995;2:S147–52.

[66] Feng R, Zhao YY, Zhu CP, Mason TJ. Enhancement of ultrasonic cavitation yield by multi-frequency sonication. Ultrason Sonochem 2002;9:231–6.

[67] Gogate PR, Shirgaonkar IZ, Sivakumar M, Senthilkumar P, Vichare NP, Pandit AB. Cavitation reactors: efficiency assessment using a model reaction. AIChE J 2001;47:2526–38.

[68] Behrend O, Ax K, Schubert H. Influence of continuous phase viscosity on emulsification by ultrasound. Ultrason Sonochem 2000;7:77–85.

[69] Sivasankar T, Moholkar VS. Mechanistic features of the sonochemical degradation of organic pollutants. AIChE J 2008;54:2206–19.

[70] Louisnard O, Lyczko N, Espitalier F, Urzedowski M, Vargas-Hernandez Y, Sanchez-Romero C. High bubble concentrations produced by ultrasounds in binary mixtures. Ultrason Sonochem 2001;8:183–9.

[71] Katekhaye SN, Gogate PR. Intensification of cavitational activity in sonochemical reactors using different additives: efficacy assessment using a model reaction. Chem Eng Process 2011;50:95–103.

[72] Lide DR. CRC handbook of chemistry and physics. 85th ed. Boca Raton: CRC Press; 2004.

[73] Behrend O, Schubert H. Influence of hydrostatic pressure and gas content on continuous ultrasound emulsification. Ultrason Sonochem 2001;8:271–6.

[74] Mahulkar AV, Riedel C, Gogate PR, Neis U, Pandit AB. Effect of dissolved gas on efficacy of sonochemical reactors for microbial cell disruption: experimental and numerical analysis. Ultrason Sonochem 2009;16:635–43.

[75] Faerman V, Mukmenev I, Shreiber I. Release of polysaccharide by sonication of cells (*Porphyridium sp.*). Acoust Phys 2009;55:270–2.

[76] Sauter C, Emin MA, Schuchmann HP, Tavman S. Influence of hydrostatic pressure and sound amplitude on the ultrasound induced dispersion and deagglomeration of nanoparticles. Ultrason Sonochem 2008;15:517–23.

[77] Sasmal S, Goud VV, Mohanty K. Ultrasound assisted lime pretreatment of lignocellulosic biomass toward bioethanol production. Energy Fuel 2012;26:3777–84.

[78] Li CZ, Yoshimoto M, Ogata H, Tsukuda N, Fukunaga K, Nakao K. Effects of ultrasonic intensity and reactor scale on kinetics of enzymatic saccharification of various waste papers in continuously irradiated stirred tanks. Ultrason Sonochem 2005;12:373–84.

[79] Gole VL, Gogate PR. Intensification of synthesis of biodiesel from non-edible oil using sequential combination of microwave and ultrasound. Fuel Process Technol 2013;106:62–9.

[80] Deshmane VG, Gogate PR, Pandit AB. Process intensification of synthesis process for medium chain glycerides using cavitation. Chem Eng J 2008;145:351–4.

[81] Cho HM, Gross AS, Chu JW. Dissecting force interactions in cellulose deconstruction reveals the required solvent versatility for overcoming biomass recalcitrance. J Am Chem Soc 2011;133:14033–41.

[82] Zhao W, Zong ZM, Lin J, Song YM, Guo XF, Yao ZS, et al. Dewaxing from stalks with petroleum ether by different methods. Energy Fuel 2007;21:1165–8.

[83] Yunus R, Salleh SF, Abdullah N, Biak DRA. Effect of ultrasonic pre-treatment on low temperature acid hydrolysis of oil palm empty fruit bunch. Bioresour Technol 2010;101:9792–6.

[84] Rezania S, Ye ZL, Berson RE. Enzymatic saccharification and viscosity of sawdust slurries following ultrasonic particle size reduction. Appl Biochem Biotechnol 2009;153:103–15.

[85] Karki B, Lamsal BP, Jung S, van Leeuwen J, Pometto III AL, Grewell D, et al. Enhancing protein and sugar release from defatted soy flakes using ultrasound technology. J Food Eng 2010;96:270–8.

[86] Pinjari DV, Pandit AB. Cavitation milling of natural cellulose to nanofibrils. Ultrason Sonochem 2010;17:845–52.

[87] Zhang QH, Benoit M, Vigier KDO, Barrault J, Jégou G, Philippe M, et al. Pretreatment of microcrystalline cellulose by ultrasounds: effect of particle size in the heterogeneously-catalyzed hydrolysis of cellulose to glucose. Green Chem 2013;15:963–9.

[88] Chen WS, Yu HP, Liu YX, Chen P, Zhang MX, Hai YF. Individualization of cellulose nanofibers from wood using high-intensity ultrasonication combined with chemical pretreatments. Carbohydr Polym 2011;83:1804–11.

[89] Filson PB, Dawson-Andoh BE. Sono-chemical preparation of cellulose nanocrystals from lignocellulose derived materials. Bioresour Technol 2009;100:2259–64.

[90] Chen WS, Yu HP, Liu YX, Hai YF, Zhang MX, Chen P. Isolation and characterization of cellulose nanofibers from four plant cellulose fibers using a chemical-ultrasonic process. Cellulose 2011;18:433–42.

[91] Chen WS, Yu HP, Liu YX. Preparation of millimeter-long cellulose I nanofibers with diameters of 30–80 nm from bamboo fibers. Carbohydr Polym 2011;86:453–61.

[92] Shi W, Li SN, Jia JF, Zhao YP. Highly efficient conversion of cellulose to bio-oil in hot-compressed water with ultrasonic pretreatment. Ind Eng Chem Res 2012;52:586–93.

[93] Lan W, Liu CF, Yue FX, Sun RC, Kennedy JF. Ultrasound-assisted dissolution of cellulose in ionic liquid. Carbohydr Polym 2011;86:672–7.

[94] Wang Q, Chen KF, Li J, Yang GH, Liu SS, Xu J. The solubility of lignin from bagasse in a 1,4-butanediol/water system. BioResources 2011;6:3034–43.

[95] Li MF, Sun SN, Xu F, Sun RC. Ultrasound-enhanced extraction of lignin from bamboo (*Neosinocalamus affinis*): characterization of the ethanol-soluble fractions. Ultrason Sonochem 2012;19:243–9.

[96] Baxi PB, Pandit AB. Using cavitation for delignification of wood. Bioresour Technol 2012;110:697–700.

[97] Moholkar VS, Warmoeskerken MMCG, Ohl CD, Prosperetti A. Mechanism of mass-transfer enhancement in textiles by ultrasound. AIChE J 2004;50:58–64.

[98] Sun RC, Sun XF, Xu XP. Effect of ultrasound on the physicochemical properties of organosolv lignins from wheat straw. J Appl Polym Sci 2002;84:2512–22.

[99] Sun RC, Sun XF, Ma XH. Effect of ultrasound on the structural and physiochemical properties of organosolv soluble hemicelluloses from wheat straw. Ultrason Sonochem 2002;9:95–101.

[100] Velmurugan R, Muthukumar K. Utilization of sugarcane bagasse for bioethanol production: sono-assisted acid hydrolysis approach. Bioresour Technol 2011;102:7119–23.

[101] García A, Alriols MG, Llano-Ponte R, Labidi J. Ultrasound-assisted fractionation of the lignocellulosic material. Bioresour Technol 2011;102:6326–30.

[102] Velmurugan R, Muthukumar K. Sono-assisted enzymatic saccharification of sugarcane bagasse for bioethanol production. Biochem Eng J 2012;63:1–9.

[103] Sindhu R, Kuttiraja M, Preeti VE, Vani S, Sukumaran RK, Binod P. A novel surfactant-assisted ultrasound pretreatment of sugarcane tops for improved enzymatic release of sugars. Bioresour Technol 2013;135:67–72.

[104] Yuan TQ, Xu F, He J, Sun RC. Structural and physico-chemical characterization of hemicelluloses from ultrasound-assisted extractions of partially delignified fast-growing poplar wood through organic solvent and alkaline solutions. Biotechnol Adv 2010;28:583–93.

[105] Yuan TQ, Sun SN, Xu F, Sun RC. Isolation and physico-chemical characterization of lignins from ultrasound irradiated fast-growing poplar wood. BioResources 2011;6:414–33.

[106] Wang XL, Fang GZ, Hu CP, Du TC. Application of ultrasonic waves in activation of microcrystalline cellulose. J Appl Polym Sci 2008;109:2762–7.

[107] Zhang YQ, Fu EH, Liang JH. Effect of ultrasonic waves on the saccharification processes of lignocellulose. Chem Eng Technol 2008;31:1510–5.

[108] Briois B, Saito T, Pétrier C, Putaux JL, Nishiyama Y, Heux L, et al. $I_\alpha \rightarrow I_\beta$ transition of cellulose under ultrasonic radiation. Cellulose 2013;20:597–603.

[109] Gonçalves AR, Schuchardt U. Hydrogenolysis of lignins – influence of the pretreatment using microwave and ultrasound irradiations. Appl Biochem Biotechnol 2002;98–100:1213–9.

[110] Ren SX, Fang GZ. Effects of ultrasonic processing on structure of alkali lignin of wheat-straw. Chem Ind For Prod 2005;25(Suppl.):82–6 [in Chinese].

[111] García A, Alriols MG, Labidi J. Evaluation of the effect of ultrasound on organosolv black liquor from olive tree pruning residues. Bioresour Technol 2012;108:155–61.

[112] García A, Erdocia X, Alriols MG, Labidi J. Effect of ultrasound treatment on the physicochemical properties of alkaline lignin. Chem Eng Process 2012;62:150–8.

[113] Zhang PF, Wang DH, Cong WL, Zhang M, Wu XR, Pei ZJ, et al. Ultrasonic vibration-assisted pelleting of cellulosic biomass for biofuel manufacturing. J Manuf Sci Eng 2011;133:011012.

[114] Wells Jr T, Kosa M, Ragauskas AJ. Polymerization of Kraft lignin via ultrasonication for high-molecular-weight applications. Ultrason Sonochem 2013;20:1463–9.

[115] Nikolić S, Mojović L, Rakin M, Pejin D, Pejin J. Ultrasound-assisted production of bioethanol by simultaneous saccharification and fermentation of corn meal. Food Chem 2010;122:216–22.

[116] Montalbo-Lomboy M, Johnson L, Khanal SK, van Leeuwen J, Grewell D. Sonication of sugary-2 corn: a potential pretreatment to enhance sugar release. Bioresour Technol 2010;101:351–8.

[117] Isono Y, Kumagai T, Watanabe T. Ultrasonic degradation of waxy rice starch. Biosci Biotechnol Biochem 1994;58:1799–802.

[118] Nitayavardhana S, Shrestha P, Rasmussen ML, Lamsal BP, van Leeuwen J, Khanal SK. Ultrasound improved ethanol fermentation from cassava chips in cassava-based ethanol plants. Bioresour Technol 2010;101:2741–7.

[119] Hernoux A, Lévêque JM, Lassi U, Molina-Boisseau S, Marais MF. Conversion of a non-water soluble potato starch waste into reducing sugars under non-conventional technologies. Carbohydr Polym 2013;92:2065–74.

[120] Imai M, Ikari K, Suzuki I. High-performance hydrolysis of cellulose using mixed cellulase species and ultrasonication pretreatment. Biochem Eng J 2004;17:79–83.

[121] Yu J, Zhang JB, He J, Liu ZD, Yu ZN. Combinations of mild physical or chemical pretreatment with biological pretreatment for enzymatic hydrolysis of rice hull. Bioresour Technol 2009;100:903–8.

[122] Velmurugan R, Muthukumar K. Ultrasound-assisted alkaline pretreatment of sugarcane bagasse for fermentable sugar production: optimization through response surface methodology. Bioresour Technol 2012;112:293–9.

[123] Pejin DJ, Mojović LV, Pejin JD, Grujić OS, Markov SL, Nikolić SB, et al. Increase in bioethanol production yield from triticale by simultaneous saccharification and fermentation with application of ultrasound. J Chem Technol Biotechnol 2012;87:170–6.

[124] Ninomiya K, Takamatsu H, Onishi A, Takahashi K, Shimizu N. Sonocatalytic-Fenton reaction for enhanced OH radical generation and its application to lignin degradation. Ultrason Sonochem 2013;20:1092–7.

[125] Ninomiya K, Kamide K, Takahashi K, Shimizu N. Enhanced enzymatic saccharification of kenaf powder after ultrasonic pretreatment in ionic liquids at room temperature. Bioresour Technol 2012;103:259–65.

[126] Yang F, Li LZ, Li Q, Tan WQ, Liu W, Xian M. Enhancement of enzymatic *in situ* saccharification of cellulose in aqueous-ionic liquid media by ultrasonic intensification. Carbohydr Polym 2010;81:311–6.

[127] Ninomiya K, Ohta A, Omote S, Ogino C, Takahashi K, Shimizu N. Combined use of completely bio-derived cholinium ionic liquids and ultrasound

irradiation for the pretreatment of lignocellulosic material to enhance enzymatic saccharification. Chem Eng J 2013;215–216:811–8.

[128] Choi JH, Kim SB. Effect of ultrasound on sulfuric acid-catalysed hydrolysis of starch. Korean J Chem Eng 1994;11:178–84.

[129] Neel PI, Gedanken A, Schwarz R, Sendersky E. Mild sonication accelerates ethanol production by yeast fermentation. Energy Fuel 2012;26:2352–6.

[130] Barton S, Bullock C, Weir D. The effects of ultrasound on the activities of some glycosidase enzymes of industrial importance. Enzyme Microb Technol 1996;18:190–4.

[131] Souza M, Mezadri ET, Zimmerman E, Leaes EX, Bassaco MM, Prá VD, et al. Evaluation of activity of a commercial amylase under ultrasound-assisted irradiation. Ultrason Sonochem 2013;20:89–94.

[132] Li Q, Ji GS, Tang YB, Gu XD, Fei JJ, Jiang HQ. Ultrasound-assisted compatible *in situ* hydrolysis of sugarcane bagasse in cellulase-aqueous-*N*-methylmorpholine-*N*-oxide system for improved saccharification. Bioresour Technol 2012;107:251–7.

[133] Xiao BY, Liu JX. Effects of various pretreatments on biohydrogen production from sewage sludge. Chin Sci Bull 2009;54:2038–44.

[134] Castrillón L, Fernández-Nava Y, Ormaechea P, Marañón E. Optimization of biogas production from cattle manure by pre-treatment with ultrasound and co-digestion with crude glycerin. Bioresour Technol 2011;102:7845–9.

[135] Wang H, Wang J, Fang Z, Wang XF, Bu HY. Enhanced bio-hydrogen production by anaerobic fermentation of apple pomace with enzyme hydrolysis. Int J Hydrogen Energy 2010;35:8303–9.

[136] Leaño EP, Babel S. Effects of pretreatment methods on cassava wastewater for biohydrogen production optimization. Renew Energy 2012;39:339–46.

[137] Sangave PC, Pandit AB. Ultrasound and enzyme assisted biodegradation of distillery wastewater. J Environ Manage 2006;80:36–46.

[138] Wu-Haan W, Burns RT, Moody LB, Hearn CJ, Grewell D. Effect of ultrasonic pretreatment on methane production potential from corn ethanol co-products. Trans ASABE 2010;53:883–90.

[139] Elbeshbishy E, Hafez H, Nakhla G. Enhancement of biohydrogen producing using ultrasonication. Int J Hydrogen Energy 2010;35:6184–93.

[140] Elbeshbishy E, Hafez H, Nakhla G. Hydrogen production using sono-bio-hydrogenator. Int J Hydrogen Energy 2011;36:1456–65.

[141] Wang ZB, Lin XM, Li PP, Zhang J, Wang SQ, Ma HL. Effects of low intensity ultrasound on cellulase pretreatment. Bioresour Technol 2012;117:222–7.

[142] Mikkola JP, Toukoniitty B, Toukoniitty E, Aumo J, Salmi T. Utilisation of on-line acoustic irradiation as a means to counter-effect catalyst deactivation in heterogeneous catalysis. Ultrason Sonochem 2004;11:233–9.

[143] Mikkola JP, Salmi T. *In-situ* ultrasonic catalyst rejuvenation in three-phase hydrogenation of xylose. Chem Eng Sci 1999;54:1583–8.

[144] Maddikeri GL, Pandit AB, Gogate PR. Intensification approaches for biodiesel synthesis from waste cooking oil: a review. Ind Eng Chem Res 2012;51:14610–28.

[145] Hanh HD, Dong NT, Okitsu K, Nishimura R, Maeda Y. Biodiesel production by esterification of oleic acid with short-chain alcohols under ultrasonic irradiation condition. Renew Energy 2009;34:780–3.

[146] Deshmane VG, Gogate PR, Pandit AB. Ultrasound-assisted synthesis of biodiesel from palm fatty acid distillate. Ind Eng Chem Res 2009;48:7923–7.

[147] Deshmane VG, Gogate PR, Pandit AB. Ultrasound assisted synthesis of *iso*-propyl esters from palm fatty acid distillate. Ultrason Sonochem 2009;16:345–50.

[148] Deng X, Fang Z, Liu YH. Ultrasonic transesterification of *Jatropha curcas L.* oil to biodiesel by a two-step process. Energy Convers Manage 2010;51:2802–7.

[149] Gole VL, Gogate PR. Intensification of synthesis of biodiesel from nonedible oils using sonochemical reactors. Ind Eng Chem Res 2012;51:11866–74.

[150] Zou HS, Lei M. Optimum process and kinetic study of *Jatropha curcas* oil pre-esterification in ultrasonical field. J Taiwan Inst Chem Eng 2012;43:730–5.

[151] Kelkar MA, Gogate PR, Pandit AB. Intensification of esterification of acids for synthesis of biodiesel using acoustic and hydrodynamic cavitation. Ultrason Sonochem 2008;15:188–94.

[152] Ji JB, Wang JL, Li YC, Yu YL, Xu ZC. Preparation of biodiesel with the help of ultrasonic and hydrodynamic cavitation. Ultrasonics 2006;44:e411–4.

[153] Chand P, Chintareddy VR, Verkade JG, Grewell D. Enhancing biodiesel production from soybean oil using ultrasonics. Energy Fuel 2010;24:2010–5.

[154] Encinar JM, González JF, Pardal A. Transesterification of castor oil under ultrasonic irradiation conditions. preliminary results. Fuel Process Technol 2012;103:9–15.

[155] Yin XL, Ma HL, You QH, Wang ZB, Chang JK. Comparison of four different enhancing methods for preparing biodiesel through transesterification of sunflower oil. Appl Energy 2012;91:320–5.

[156] Stavarache C, Vinatoru M, Nishimura R, Maeda Y. Conversion of vegetable oil to biodiesel using ultrasonic irradiation. Chem Lett 2003;32:716–7.

[157] Mahamuni NN, Adewuyi YG. Application of Taguchi method to investigate the effects of process parameters on the transesterification of soybean oil using high frequency ultrasound. Energy Fuel 2010;24:2120–6.

[158] Stavarache C, Vinatoru M, Nishimura R, Maeda Y. Fatty acids methyl esters from vegetable oil by means of ultrasonic energy. Ultrason Sonochem 2005;12:367–72.

[159] Georgogianni KG, Katsoulidis AK, Pomonis PJ, Manos G, Kontominas MG. Transesterification of rapeseed oil for the production of biodiesel using homogeneous and heterogeneous catalysis. Fuel Process Technol 2009;90:1016–22.

[160] Hingu SM, Gogate PR, Rathod VK. Synthesis of biodiesel from waste cooking oil using sonochemical reactors. Ultrason Sonochem 2010;17:827–32.

[161] Manh DV, Chen YH, Chang CC, Chang CY, Minh CV, Hanh HD. Parameter evaluation of biodiesel production from unblended and blended Tung oils *via* ultrasound-assisted process. J Taiwan Inst Chem Eng 2012;43:368–73.

[162] Komers K, Stloukal R, Machek J, Skopal F. Biodiesel from rapeseed oil, methanol and KOH. 3. Analysis of composition of actual reaction mixture. Eur J Lipid Sci Technol 2001;103:363–71.

[163] Stavarache C, Vinatoru M, Maeda Y. Aspects of ultrasonically assisted transesterification of various vegetable oils with methanol. Ultrason Sonochem 2007;14:380–6.

[164] Hanh HD, Dong NT, Okitsu K, Nishimura R, Maeda Y. Biodiesel production through transesterification of triolein with various alcohols in an ultrasonic field. Renew Energy 2009;34:766–8.

[165] Mahamuni NN, Adewuyi YG. Optimization of the synthesis of biodiesel *via* ultrasound-enhanced base-catalyzed transesterification of soybean oil using a multifrequency ultrasonic reactor. Energy Fuel 2009;23:2757–66.

[166] Ye XJ, Fernando S, Wilson W, Singh A. Application of amphiphilic catalysts, ultrasonication, and nanoemulsions for biodiesel production process. Chem Eng Technol 2007;30:1481–7.

[167] Hsiao MC, Lin CC, Chang YH, Chen LC. Ultrasonic mixing and closed microwave irradiation-assisted transesterification of soybean oil. Fuel 2010;89:3618–22.

[168] Mootabadi H, Salamatinia B, Bhatia S, Abdullah AZ. Ultrasonic-assisted biodiesel production process from palm oil using alkaline earth metal oxides as the heterogeneous catalysts. Fuel 2010;89:1818–25.

[169] Verziu M, Coman SM, Richards R, Parvulescu VI. Transesterification of vegetable oils over CaO catalysts. Catal Today 2011;167:64–70.

[170] Deng X, Fang Z, Liu YH, Yu CL. Production of biodiesel from *Jatropha* oil catalyzed by nanosized solid basic catalyst. Energy 2011;36:777–84.

[171] Georgogianni KG, Katsoulidis AP, Pomonis PJ, Kontominas MG. Transesterification of soybean frying oil to biodiesel using heterogeneous catalysts. Fuel Process Technol 2009;90:671–6.

[172] Kumar D, Kumar G, Poonam, Singh CP. Ultrasonic-assisted transesterification of *Jatropha curcus* oil using solid catalyst, Na/SiO_2. Ultrason Sonochem 2010;17:839–44.

[173] Chen X, Qian WW, Lv XP, Han PF. Preparation of biodiesel catalysed by KF/CaO with ultrasound. Nat Prod Res 2012;26:1249–56.

[174] Ren QG, Yan J, Huang GH, Qiu TQ, Yang RF, Fan XD. Nano KF/γ-Al_2O_3 catalyzed transesterification of soybean oil for biodiesel production by using ultrasonic irradiation. J Cent South Univ (Sci Technol) 2010;41:883–9 [in Chinese].

[175] López DE, Goodwin Jr JG, Bruce DA, Lotero E. Transesterification of triacetin with methanol on solid acid and base catalysts. Appl Catal A 2005;295:97–105.

[176] Koberg M, Gedanken A. Direct transesterification of castor and *Jatropha* seeds for FAME production by microwave and ultrasound radiation using a SrO catalyst. Bioenergy Res 2012;5:958–68.

[177] Salamatinia B, Abdullah AZ, Bhatia S. Quality evaluation of biodiesel produced through ultrasound-assisted heterogeneous catalytic system. Fuel Process Technol 2012;97:1–8.

[178] Badday AS, Abdullah AZ, Lee KT. Ultrasound-assisted transesterification of crude *Jatropha* oil using alumina-supported heteropolyacid catalyst. Appl Energy 2013;105:380–8.

[179] Liu YX, Jin QZ, Shan L, Liu YF, Shen W, Wang XG. The effect of ultrasound on lipase-catalyzed hydrolysis of soy oil in solvent-free system. Ultrason Sonochem 2008;15:402–7.

[180] Fiametti KG, Sychoski MM, Cesaro AD, Furigo Jr A, Bretanha LC, Pereira CMP, et al. Ultrasound irradiation promoted efficient solvent-free lipase-catalyzed production of mono- and diacylglycerols from olive oil. Ultrason Sonochem 2011;18:981–7.

[181] Kumar G, Kumar D, Poonam, Johari R, Singh CP. Enzymatic transesterification of *Jatropha curcas* oil assisted by ultrasonication. Ultrason Sonochem 2011;18:923–7.

[182] Yu DH, Tian L, Wu H, Wang S, Wang Y, Ma DX, et al. Ultrasonic irradiation with vibration for biodiesel production from soybean oil by *Novozym 435*. Process Biochem 2010;45:519–25.

[183] Batistella L, Lerin LA, Brugnerotto P, Danielli AJ, Trentin CM, Popiolski A, et al. Ultrasound-assisted lipase-catalyzed transesterification of soybean oil in organic solvent system. Ultrason Sonochem 2012;19:452–8.

[184] Tupufia SC, Jeon YJ, Marquis C, Adesina AA, Rogers PL. Enzymatic conversion of coconut oil for biodiesel production. Fuel Process Technol 2013;106:721–6.

[185] Gharat N, Rathod VK. Ultrasound assisted enzyme catalyzed transesterification of waste cooking oil with dimethyl carbonate. Ultrason Sonochem 2013;20:900–5.

[186] Shah S, Gupta MN. The effect of ultrasonic pre-treatment on the catalytic activity of lipases in aqueous and non-aqueous media. Chem Cent J 2008;2:1–9.

[187] Minami E, Saka S. Kinetics of hydrolysis and methyl esterification for biodiesel production in two-step supercritical methanol process. Fuel 2006;85:2479–83.

[188] Lee JS, Saka S. Biodiesel production by heterogeneous catalysts and supercritical technologies. Bioresour Technol 2010;101:7191–200.

[189] Biktashev SA, Usmanov RA, Gabitov RR, Gazizov RA, Gumerov FM, Gabitov FR, et al. Transesterification of rapeseed and palm oils in supercritical methanol and ethanol. Biomass Bioenergy 2011;35:2999–3011.

[190] Gobikrishnan S, Park JH, Park SH, Indrawan N, Rahman SF, Park DH. Sonication-assisted production of biodiesel using soybean oil and supercritical methanol. Bioprocess Biosyst Eng 2013;36:705–12.

[191] Khanna S, Goyal A, Moholkar V. Microbial conversion of glycerol: present status and future prospects. Crit Rev Biotechnol 2012;32:235–62.

[192] Pagliaro M, Ciriminna R, Kimura H, Rossi M, Pina CD. From glycerol to value-added products. Angew Chem Int Ed 2007;46:4434–40.

[193] Khanna S, Jaiswal S, Goyal A, Moholkar VS. Ultrasound enhanced bioconversion of glycerol by *Clostridium pasteurianum*: a mechanistic investigation. Chem Eng J 2012;200–202:416–25.

[194] Khanna S, Goyal A, Moholkar VS. Mechanistic investigation of ultrasonic enhancement of glycerol bioconversion by immobilized *Clostridium pasteurianum* on silica support. Biotechnol Bioeng 2013;110:1637–45.

[195] Bolívar-Diaz CL, Calvino-Casilda V, Rubio-Marcos F, Fernández JF, Bañares MA. New concepts for process intensification in the conversion of glycerol carbonate to glycidol. Appl Catal B 2013;129:575–9.

[196] Ramachandran KB, Al-Zuhair S, Fong CS, Gak CW. Kinetic study on hydrolysis of oils by lipase with ultrasonic emulsification. Biochem Eng J 2006;32:19–24.

[197] Wu P, Yang Y, Colucci JA, Grulke EA. Effect of ultrasonication on droplet size in biodiesel mixtures. J Am Oil Chem Soc 2007;84:877–84.

[198] Parkar PA, Choudhary HA, Moholkar VS. Mechanistic and kinetic investigations in ultrasound assisted acid catalyzed biodiesel synthesis. Chem Eng J 2012;187:248–60.

[199] Leong TSH, Wooster TJ, Kentish SE, Ashokkumar M. Minimising oil droplet size using ultrasonic emulsification. Ultrason Sonochem 2009;16:721–7.

[200] Kalva A, Sivasankar T, Moholkar VS. Physical mechanism of ultrasound-assisted synthesis of biodiesel. Ind Eng Chem Res 2009;48:534–44.

[201] Hu Q, Sommerfeld M, Jarvis E, Ghirardi M, Posewitz M, Seibert M, et al. Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. Plant J 2008;54:621–39.

[202] Chisti Y. Biodiesel from microalgae. Biotechnol Adv 2007;25:294–306.

[203] Francko DA, Taylor SR, Thomas BJ, McIntosh D. Effect of low-dose ultrasonic treatment on physiological variables in *Anabaena flos-aquae* and *Selenastrum capricornutum*. Biotechnol Lett 1990;12:219–24.

[204] Bozhkova M, Dencheva A. Ultrasound influence on *Scenedesmus acutus* productivity and protein content. Bulg J Plant Physiol 1995;21:58–67.

[205] Al-Hamdani S, Burnett C, Durrant G. Effect of low-dose ultrasonic treatment on *Spirulina maxima*. Aquac Eng 1998;19:17–28.

[206] Angerbauer C, Siebenhofer M, Mittelbach M, Guebitz GM. Conversion of sewage sludge into lipids by *Lipomyces starkeyi* for biodiesel production. Bioresour Technol 2008;99:3051–6.

[207] Uduman N, Qi Y, Danquah MK, Forde GM, Hoadley A. Dewatering of microalgal cultures: a major bottleneck to algae-based fuels. J Renew Sustain Energy 2010;2:012701–15.

[208] Bosma R, van Spronsen WA, Tramper J, Wijffels RH. Ultrasound, a new separation technique to harvest microalgae. J Appl Phycol 2003;15:143–53.

[209] Leckey CC, Hinders M. Viscous effects in the acoustic manipulation of algae for biofuel production. J Appl Phycol 2012;24:145–56.

[210] Broekman S, Pohlmann O, Beardwood ES, de Meulenaer EC. Ultrasonic treatment for microbiological control of water systems. Ultrason Sonochem 2010;17:1041–8.

[211] Rajasekhar P, Fan LH, Nguyen T, Roddick FA. A review of the use of sonication to control cyanobacterial blooms. Water Res 2012;46:4319–29.

[212] Cheng CH, Du TB, Pi HC, Jang SM, Lin YH, Lee HT. Comparative study of lipid extraction from microalgae by organic solvent and supercritical CO_2. Bioresour Technol 2011;102:10151–3.

[213] Woods L, Riccobono M, Mehan N, Hestekin J, Beitle R. Synergistic effect of abrasive and sonication for release of carbohydrate and protein from algae. Sep Sci Technol 2011;46:601–4.

[214] Araujo GS, Matos LJBL, Gonçalves LRB, Fernandes FAN, Farias WRL. Bioprospecting for oil producing microalgal strains: evaluation of oil and biomass production for ten microalgal strains. Bioresour Technol 2011;102:5248–50.

[215] Šoštarič M, Klinar D, Bricelj M, Golob J, Berovič M, Likozar B. Growth, lipid extraction and thermal degradation of the microalga *Chlorella vulgaris*. New Biotechnol 2012;29:325–31.

[216] Crespo MOP, Cam D, Gagni S, Lombardi N, Yusty MAL. Extraction of hydrocarbons from seaweed samples using sonication and microwave-assisted extraction: a comparative study. J Chromatogr Sci 2006;44:615–8.

[217] Kumari P, Reddy CRK, Jha B. Comparative evaluation and selection of a method for lipid and fatty acid extraction from macroalgae. Anal Biochem 2011;415:134–44.

[218] Zhao GL, Chen X, Wang L, Zhou SX, Feng HX, Chen WN, et al. Ultrasound assisted extraction of carbohydrates from microalgae as feedstock for yeast fermentation. Bioresour Technol 2013;128:337–44.

[219] Benavides J, Rito-Palomares M. Simplified two-stage method to *B-phycoerythrin* recovery from *Porphyridium cruentum*. J Chromatogr B 2006;844:39–44.

[220] Rogers JE, Marcovich D. A simple method for the extraction and quantification of photopigments from *Symbiodinium spp*. J Exp Mar Biol Ecol 2007;353:191–7.

[221] Pasquet V, Chérouvrier JR, Farhat F, Thiéry V, Piot JM, Bérard JB, et al. Study on the microalgal pigments extraction process: performance of microwave assisted extraction. Process Biochem 2011;46:59–67.

[222] Lee JY, Yoo C, Jun SY, Ahn CY, Oh HM. Comparison of several methods for effective lipid extraction from microalgae. Bioresour Technol 2010;101:S75–7.

[223] D'Oca MGM, Viêgas CV, Lemões JS, Miyasaki EK, Morón-Villarreyes JA, Primel EG, et al. Production of FAMEs from several microalgal lipidic extracts and direct transesterification of the *Chlorella pyrenoidosa*. Biomass Bioenergy 2011;35:1533–8.

[224] Prabakaran P, Ravindran AD. A comparative study on effective cell disruption methods for lipid extraction from microalgae. Lett Appl Microbiol 2011;53:150–4.

[225] Adam F, Abert-Vian M, Peltier G, Chemat F. "Solvent-free" ultrasound-assisted extraction of lipids from fresh microalgae cells: a green, clean and scalable process. Bioresour Technol 2012;114:457–65.

[226] Araujo GS, Matos LJBL, Fernandes JO, Cartaxo SJM, Gonçalves LRB, Fernandes FAN, et al. Extraction of lipids from microalgae by ultrasound application: prospection of the optimal extraction method. Ultrason Sonochem 2013;20:95–8.

[227] Wang G, Wang T. Lipid and biomass distribution and recovery from two microalgae by aqueous and alcohol processing. J Am Oil Chem Soc 2012;89:335–45.

[228] Nowotarski KKPM, Joyce EM, Mason TJ. Ultrasonic disruption of algae cells. AIP Conf Proc 2012;1433:237–40.

[229] Yun YM, Jung KW, Kim DH, Oh YK, Cho SK, Shin HS. Optimization of dark fermentative H_2 production from microalgal biomass by combined (acid + ultrasonic) pretreatment. Bioresour Technol 2013;141:220–6.

[230] Nguyen TAD, Kim KR, Nguyen MT, Kim MS, Kim D, Sim SJ. Enhancement of fermentative hydrogen production from green algal biomass of *Thermotoga neapolitana* by various pretreatment methods. Int J Hydrogen Energy 2010;35:13035–40.

[231] Choi JA, Hwang JH, Dempsey BA, Abou-Shanab RAI, Min B, Song H, et al. Enhancement of fermentative bioenergy (ethanol/hydrogen) production using ultrasonication of *Scenedesmus obliquus* YSW15 cultivated in swine wastewater effluent. Energy Environ Sci 2011;4:3513–20.

[232] González-Fernández C, Sialve B, Bernet N, Steyer JP. Comparison of ultrasound and thermal pretreatment of *Scenedesmus* biomass on methane production. Bioresour Technol 2012;110:610–6.

[233] Alzate ME, Muñoz R, Rogalla F, Fdz-Polanco F, Pérez-Elvira SI. Biochemical methane potential of microalgae: influence of substrate to inoculum ratio, biomass concentration and pretreatment. Bioresour Technol 2012;123:488–94.

[234] Liang KH, Zhang QH, Cong W. Enzyme-assisted aqueous extraction of lipid from microalgae. J Agric Food Chem 2012;60:11771–6.

[235] Zhou CS, Ma HL. Ultrasonic degradation of polysaccharide from a red algae (*Porphyra yezoensis*). J Agric Food Chem 2006;54:2223–8.

[236] Zhou CS, Yu XJ, Zhang YZ, He RH, Ma HL. Ultrasonic degradation, purification and analysis of structure and antioxidant activity of polysaccharide from *Porphyra yezoensis* Udea. Carbohydr Polym 2012;87:2046–51.

[237] Koberg M, Cohen M, Ben-Amotz A, Gedanken A. Bio-diesel production directly from the microalgae biomass of *Nannochloropsis* by microwave and ultrasound radiation. Bioresour Technol 2011;102:4265–9.

[238] Sivaramakrishnan R, Muthukumar K. Production of methyl ester from *Oedogonium sp.* oil using immobilized isolated novel *Bacillus sp.* lipase. Energy Fuel 2012;26:6387–92.

[239] Tran DT, Chen CL, Chang JS. Effect of solvents and oil content on direct transesterification of wet oil-bearing microalgal biomass of *Chlorella vulgaris* ESP-31 for biodiesel synthesis using immobilized lipase as the biocatalyst. Bioresour Technol 2013;135:213–21.

[240] Jeon BH, Choi JA, Kim HC, Hwang JH, Abou-Shanab RAI, Dempsey BA, et al. Ultrasonic disintegration of microalgal biomass and consequent improvement of bioaccessibility/bioavailability in microbial fermentation. Biotechnol Biofuel 2013;6:37.

[241] Gerde JA, Montalbo-Lomboy M, Yao LX, Grewell D, Wang T. Evaluation of microalgae cell disruption by ultrasonic treatment. Bioresour Technol 2012;125:175–81.

[242] McMillan JR, Watson IA, Ali M, Jaafar W. Evaluation and comparison of algal cell disruption methods: microwave, waterbath, blender, ultrasonic and laser treatment. Appl Energy 2013;103:128–34.

[243] Liang W, Wang TJ, Zhang Q, Wang L, Xu Y, Wu CZ. Ultrasonic emulsification characteristics of aqueous solution of bio-oil from fast pyrolysis of biomass and diesel. J Fuel Chem Technol 2009;37:684–9 [in Chinese].

[244] Li YP, Wang TJ, Liang W, Wu CZ, Ma LL, Zhang Q, et al. Ultrasonic preparation of emulsions derived from aqueous bio-oil fraction and 0# diesel and combustion characteristics in diesel generator. Energy Fuel 2010;24:1987–95.

[245] Du MT, Huang YC, Shang S, Wang P, Wang L, Guo HJ. Study on optimum HLB value and physicochemical properties of diesel and bio-oil emulsions. J Fuel Chem Technol 2009;37:679–83 [in Chinese].

[246] Huang YC, Shang S, Wang L, Du MT, Guo HJ. Application of biomass pyrolysis oil to direct injection diesel engine. Trans CSAE 2010;26:290–4 [in Chinese].

[247] Nii S, Kikumoto S, Tokuyama H. Quantitative approach to ultrasonic emulsion separation. Ultrason Sonochem 2009;16:145–9.

[248] Pangu GD, Feke DL. Acoustically aided separation of oil droplets from aqueous emulsions. Chem Eng Sci 2004;59:3183–93.

[249] Kaatze U, Hushcha TO, Eggers F. Ultrasonic broadband spectrometry of liquids: a research tool in pure and applied chemistry and chemical physics. J Solution Chem 2000;29:299–368.

[250] Kudryashov E, Smyth C, O'Driscoll B, Buckin V. High-resolution ultrasonic spectroscopy for analysis of chemical reactions in real time. Spectroscopy 2003;18:26–32.

[251] Elmehdi HM, Page JH, Scanlon MG. Monitoring dough fermentation using acoustic waves. Food Bioprod Process 2003;81:217–23.

[252] Resa P, Kudryashov E, Buckin V. Direct real time monitoring of cellulose saccharification using high-resolution ultrasonic spectroscopy. New Biotechnol 2009;25(Suppl.):S264–5.
[253] Challis RE, Povey MJW, Mather ML, Holmes AK. Ultrasound techniques for characterizing colloidal dispersions. Rep Prog Phys 2005;68:1541–637.
[254] Wang XZ, Liu L, Li RF, Tweedie RJ, Primrose K, Corbett J, et al. Online characterisation of nanoparticle suspensions using dynamic light scattering, ultrasound spectroscopy and process tomography. Chem Eng Res Des 2009;87:874–84.
[255] Behrends R, Cowman MK, Eggers F, Eyring EM, Kaatze U, Majewski J, et al. Ultrasonic relaxation and fast chemical kinetics of some carbohydrate aqueous solutions. J Am Chem Soc 1997;119:2182–6.
[256] Stenger J, Cowman M, Eggers F, Eyring EM, Kaatze U, Petrucci S. Molecular dynamics and kinetics of monosaccharides in solution. A broadband ultrasonic relaxation study. J Phys Chem B 2000;104:4782–90.
[257] Figueiredo MKK, Costa-Felix RPB, Maggi LE, Alvarenga AV, Romeiro GA. Biofuel ethanol adulteration detection using an ultrasonic measurement method. Fuel 2012;91:209–12.
[258] Tat ME, Van Gerpen JH. Effect of temperature and pressure on the speed of sound and isentropic bulk modulus of mixtures of biodiesel and diesel fuel. J Am Oil Chem Soc 2003;80:1127–30.
[259] Tat ME, Van Gerpen JH. Speed of sound and isentropic bulk modulus of alkyl monoesters at elevated temperatures and pressures. J Am Oil Chem Soc 2003;80:1249–56.
[260] Chanamai R, Coupland JN, McClements DJ. Effect of temperature on the ultrasonic properties of oil-in-water emulsions. Colloid Surf A 1998;139:241–50.
[261] Parveen S, Shukla D, Singh S, Singh KP, Gupta M, Shukla JP. Ultrasonic velocity, density, viscosity and their excess parameters of the binary mixtures of tetrahydrofuran with methanol and *o*-cresol at varying temperatures. Appl Acoust 2009;70:507–13.
[262] Yucel U, Coupland JN. Ultrasonic attenuation measurements of the mixing, agglomeration, and sedimentation of sucrose crystals suspended in oil. J Am Oil Chem Soc 2011;88:33–8.
[263] Benedito J, García-Pérez JV, Dobarganes MC, Mulet A. Rapid evaluation of frying oil degradation using ultrasonic technology. Food Res Int 2007;40:406–14.
[264] Resa P, Elvira L, de Espinosa FM, González R, Barcenilla J. On-line ultrasonic velocity monitoring of alcoholic fermentation kinetics. Bioprocess Biosyst Eng 2009;32:321–31.
[265] Resa P, Buckin V. Ultrasonic analysis of kinetic mechanism of hydrolysis of cellobiose by β-glucosidase. Anal Biochem 2011;415:1–11.
[266] Koc AB. Ultrasonic monitoring of glycerol settling during transesterification of soybean oil. Bioresour Technol 2009;100:19–24.
[267] Koc AB, McKenzie EH. Effects of ultrasonication on glycerin separation during transesterification of soybean oil. Fuel Process Technol 2010;91:743–8.
[268] Cents AHG, Kersten SRA, Brilman DWF. Gas-phase RTD measurement in gas and gas-solids reactors using ultrasound. Ind Eng Chem Res 2003;42:5506–15.
[269] Boyer C, Duquenne AM, Wild G. Measuring techniques in gas-liquid and gas-liquid-solid reactors. Chem Eng Sci 2002;57:3185–215.
[270] Marda JR, DiBenedetto J, McKibben S, Evans RJ, Czernik S, French RJ, et al. Non-catalytic partial oxidation of bio-oil to synthesis gas for distributed hydrogen production. Int J Hydrogen Energy 2009;34:8519–34.
[271] Finch KBH, Richards RM, Richel A, Medvedovici AV, Gheorghe NG, Verziu M, et al. Catalytic hydroprocessing of lignin under thermal and ultrasound conditions. Catal Today 2012;196:3–10.
[272] Brochette-Lemoine S, Trombotto S, Joannard D, Descotes G, Bouchu A, Queneau Y. Ultrasound in carbohydrate chemistry: sonophysical glucose oligomerisation and sonocatalysed sucrose oxidation. Ultrason Sonochem 2000;7:157–61.
[273] Sivasankar T, Moholkar VS. Mechanistic approach to intensification of sonochemical degradation of phenol. Chem Eng J 2009;149:57–69.
[274] Kobayashi D, Sano K, Takeuchi Y, Terasaka K. Effect of irradiation distance on degradation of phenol using indirect ultrasonic irradiation method. Ultrason Sonochem 2011;18:1205–10.
[275] Kidak R, Ince NH. Effects of operating parameters on sonochemical decomposition of phenol. J Hazard Mater 2006;B137:1453–7.
[276] Jie MSFLK, Kalluri P. Ultrasound-assisted oxidative cleavage of acetylenic and ethylenic bonds in unsaturated fatty esters with potassium permanganate. Lipids 1996;31:1299–301.
[277] Gogate PR. Cavitational reactors for process intensification of chemical processing applications: a critical review. Chem Eng Process 2008;47:515–27.
[278] Yu FW, Ji JB, Liu HZ. Application of ultrasound in catalytic process. Appl Acoust 2002;21:40–5 [in Chinese].
[279] Climent MJ, Corma A, Iborra S, Epping K, Velty A. Increasing the basicity and catalytic activity of hydrotalcites by different synthesis procedures. J Catal 2004;225:316–26.
[280] Boffito DC, Crocellà V, Pirola C, Neppolian B, Cerrato G, Ashokkumar M, et al. Ultrasonic enhancement of the acidity, surface area and free fatty acids esterification catalytic activity of sulphated ZrO_2-TiO_2 systems. J Catal 2013;297:17–26.
[281] Sivakumar M, Gedanken A, Zhong ZY, Chen LW. Acoustic cavitation-an efficient energetic tool to synthesize nanosized CuO-ZrO_2 catalysts with a mesoporous distribution. New J Chem 2006;30:102–7.
[282] Radziuk D, Möhwald H, Shchukin D. Ultrasonic activation of platinum catalysts. J Phys Chem C 2008;112:19257–62.
[283] Chimentão RJ, Abelló S, Medina F, Llorca J, Sueiras JE, Cesteros Y, et al. Defect-induced strategies for the creation of highly active hydrotalcites in base-catalyzed reactions. J Catal 2007;252:249–57.
[284] Sivakumar M, Towata A, Yasui K, Tuziuti T, Kozuka T, Iida Y. Dependence of sonochemical parameters on the platinization of rutile titania – an observation of a pronounced increase in photocatalytic efficiencies. Ultrason Sonochem 2010;17:621–7.
[285] Sivakumar M, Towata A, Yasui K, Tuziuti T, Kozuka T, Tsujimoto M, et al. Fabrication of nanosized Pt on rutile TiO_2 using a standing wave sonochemical reactor (SWSR) – observation of an enhanced catalytic oxidation of CO. Ultrason Sonochem 2010;17:213–8.
[286] Liu XF, Yu YL, Lu XH, Xu ZC, Ji JB. Research progress of ultrasound application to adsorption/desorption process. Chem Ind Eng Prog 2006;25:639–45 [in Chinese].
[287] Schueller BS, Yang RT. Ultrasound enhanced adsorption and desorption of phenol on activated carbon and polymeric resin. Ind Eng Chem Res 2001;40:4912–8.
[288] Breitbach M, Bathen D. Influence of ultrasound on adsorption processes. Ultrason Sonochem 2001;8:277–83.
[289] Ando T, Sumi S, Kawate T, Ichihara J, Hanafusa T. Sonochemical switching of reaction pathways in solid-liquid two-phase reactions. J Chem Soc Chem Commun 1984;7:439–40.
[290] Suslick KS, Casadonte DJ. Heterogeneous sonocatalysis with nickel powder. J Am Chem Soc 1987;109:3459–61.
[291] Zhang Y, Stanciulescu M, Ikura M. Rapid transesterification of soybean oil with phase transfer catalysts. Appl Catal A 2009;366:176–83.
[292] Crossley S, Faria J, Shen M, Resasco DE. Solid nanoparticles that catalyze biofuel upgrade reaction at the water/oil interface. Science 2010;327:68–72.
[293] Spengler JF, Coakley WT, Christensen KT. Microstreaming effects on particle concentration in an ultrasonic standing wave. AIChE J 2003;49:2773–82.
[294] O'Donnell CP, Tiwari BK, Bourke P, Cullen PJ. Effect of ultrasonic processing on food enzymes of industrial importance. Trends Food Sci Technol 2010;21:358–67.
[295] Lopez P, Sala FJ, de la Fuente JL, Condon S, Raso J, Burgos J. Inactivation of peroxidase, lipoxygenase, and polyphenol oxidase by manothermosonication. J Agric Food Chem 1994;42:252–6.
[296] Özbek B, Ülgen KÖ. The stability of enzymes after sonication. Process Biochem 2000;35:1037–43.
[297] Riesz P, Kondo T. Free radical formation induced by ultrasound and its biological implications. Free Radic Biol Med 1992;13:247–70.
[298] Gupta MN. Enzyme function in organic solvents. Eur J Biochem 1992;203:25–32.
[299] Klibanov AM. Improving enzymes by using them in organic solvents. Nature 2001;409:241–6.
[300] Huang RL, Su RX, Qi W, He ZM. Bioconversion of lignocellulose into bioethanol: process intensification and mechanism research. Bioenergy Res 2011;4:225–45.
[301] Balasubramanian RK, Doan TTY, Obbard JP. Factors affecting cellular lipid extraction from marine microalgae. Chem Eng J 2013;215–216:929–36.
[302] Karki B, Maurer D, Jung S. Efficiency of pretreatments for optimal enzymatic saccharification of soybean fiber. Bioresour Technol 2011;102:6522–8.

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