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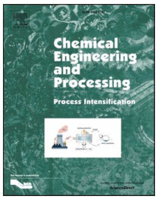

Enzymatic hydrolysis of waste fats, oils and greases (FOGs): Status, prospective, and process intensification alternatives

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ABSTRACT

The increasing global generation of waste fats, oils, and greases (FOGs) is resulting in a cascade of economic, environmental, and public health issues as mismanagement is a common practice worldwide. Hence industrial exploitation and valorization is required towards a circular economy within the oleochemical industry. FOGs suitable for further oleochemical exploitation include used cooking oils, trap greases, mill effluents, rendering fats, spent earths from filtration and bleaching, deodorization distillates, soapstocks, and sewage sludges, among others. However main challenge for FOGs harnessing are their heterogeneity and the high content of impurities, acidity, and water. Alternatively, waste FOGs could be subjected to complete hydrolysis to recover free fatty acids (FFAs), which are widely used oleochemical feedstocks. Different catalysts have been explored for this process, however, lipases are of particular interest because they are able to work on low-quality feedstocks under milder operating conditions. This paper reviews most recent investigations on the enzymatic hydrolysis of waste FOGs, describing main processes characteristics, operating conditions, challenges, current attempts to improve enzymes performance, the use of combined chemical processes to improve feedstock utilization, and the implementation of intensified processing technologies. Potential for industrial implementation and future research possibilities in the process intensification field are identified.

1. Introduction

Vegetable oils and animal fats are the main feedstocks of the oleochemical industry, and they are widely used in the manufacture of a large variety of derivatives [1]. Oils and fats are mainly composed of triacylglycerides of fatty acids having different chain lengths and saturation character, and their concentration and nature are mainly dictated by the corresponding biobased source. While triacylglycerides (TAGs) can be directly used as feedstock in many processes, derived basic oleochemicals such as fatty acids and their methyl esters are preferred as raw materials because they can be more easily handled, fractionated, and split according to their carbon chain length and their saturated-unsaturated nature [2]. Among basic oleochemicals, free fatty acids (FFAs) are of major importance as they constitute a great portion of the renewable materials currently used in the chemical industry [3]. As described in Fig. 1, FFAs are mainly produced by sequential-parallel hydrolysis of TAGs, and they are used to obtain a large variety of molecules that are difficult to produce via petrochemical pathways [4]. These include soaps, surfactants, lubricants, plasticizers, and many other commodity, fine and specialty chemicals [5]. Among the different FFAs, polyunsaturated fatty acids (PUFAs) are of special interest because they have enhanced nutritional value for food/feed applications, and they are also suitable feedstocks for biopolymers production, which are currently under high and increasing demand [6,7].

Even tough biobased and renewable resources have gained significant use in the chemical industry, the awareness on the associated life cycle impacts of agricultural-based raw materials is necessary. A better understanding of the water-energy-food security nexus have redirected the attention to reclaiming and exploitation of biobased residues [8]. In the case of the oleochemical industry, there is an increasing interest for exploiting waste fats oils and greases (FOGs). These mainly correspond to food post consumption residues mainly from large urban centers and to industrial and agricultural wastes. On one hand, urban waste lipids correspond to inedible fats and oils from animal rendering, grease traps, food residues, and used cooking oils, among others [9]. On the other hand, industrial and agricultural waste lipids include rancid oils, spent

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filtering earths, soapstocks, mill effluents, deodorization distillates, etc. [10]. Currently, millions of tons of such waste lipids are generated and mishandled worldwide, causing a cascade of environmental, health, and economic impacts, and also losing their value as oleochemical resources. For instance, the wrong disposal of used cooking oils and trap greases in the sewage provokes accumulation and clogging, causing overflows, floodings during rainy seasons, rodents and vectors proliferation, in addition to water pollution and ecosystems damage. All this represents large expenditures of public funds on corrective maintenance. Likewise, waste lipids disposal on solid residues generates a waterproofing layer which hinders the biodegradation of biomass in landfills [11].

In spite of the potential benefits of using waste FOGs as industrial feedstock, there are still many logistics, supply chain and technical challenges to overcome [12]. These residues are characterized by a higher heterogeneity, with a higher content of impurities and acidity [13], thereby a variety of pre-treatment processes are required for their transformation into suitable oleochemical feedstocks [14]. Currently, a major goal of pretreatment is the reduction of moisture and acidity to fulfill the required specifications for biofuels production, as this is the most common valorization route implemented at the industrial scale [15]. Similar to edible oils and fats refining, FFAs removal from waste lipids is generally accomplished by physical and/or chemical processes [16,17]. However, as the acidity content can be large, pretreatment can be cumbersome, costly and residues-intensive. Alternatively, recognizing the extensive use of fatty acids, and instead of reclaiming and refining only the TAGs fraction of waste FOGs, these could be subjected to hydrolysis to enable the complete recovery of FFAs for further oleochemical use.

Currently, industrial FFAs are commonly manufactured by hydrolysis of edible oils and fats under extreme conditions via highly energy-intensive processes (e.g. Colgate-Emery, Eisenlohr) [18]. These processes involve the acid-catalyzed or uncatalyzed reaction with water under high temperatures and pressures (i.e., around 250 °C and 50 bar), and such conditions are required to enhance miscibility between oil and water, to reduce oil viscosity, to overcome equilibrium limitations and to achieve fast kinetics. In addition to requiring costly equipment and high operating expenses, these extreme conditions also trigger undesired side reactions such as the polymerization of valuable PUFAs [3,19,20]. In this regard, catalytic hydrolysis under milder conditions is a greener alternative to the current industrial processes. While reducing the energy of activation of reaction, the use of heterogeneous catalysts could also mitigate equipment corrosion, facilitate separation, and production of undesired products [3,19,21]. Additionally, operating under milder conditions would enable exploitation of low quality and waste oleochemical feedstocks, limiting a further decomposition of such materials [22].

Among the variety of active materials that can be used to promote the hydrolysis of low-quality feedstocks, bioderived enzymatic catalysts have attracted attention recently due to their high activity under milder conditions, high regioselectivity, and substrate specificity [23]. Among the variety of enzymes used to promote hydrolysis of waste lipids, lipases belong to a special group that exhibit high activity even in hydrophobic media. This feature gives lipases the ability to selectively catalyze different reactions beside hydrolysis. This includes esterification, interesterification, alcoholysis, acidolysis, and aminolysis [24]. Nonetheless, despite their high activity and selectivity, the extended use of lipases in the industry is still limited mainly due to their high costs compared with typical homogeneous and heterogeneous catalysts. As a result, researchers have focused on developing new and more specific lipases, with higher activity, more resilience to the reactive media, with higher stability to operating conditions, and suitable for recycling and reuse. An effort in this direction is the use of immobilized lipases, which

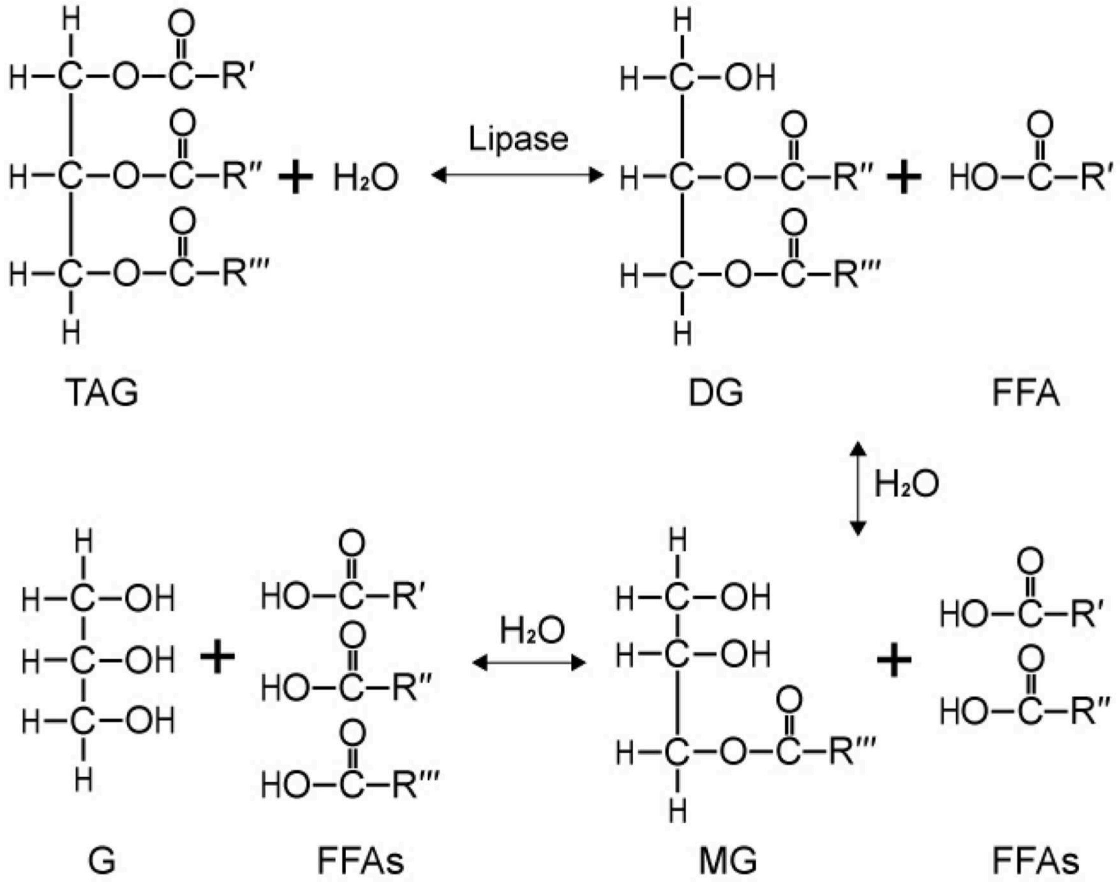

Fig. 1. Reaction scheme for the hydrolysis of triacylglycerides (TAG) into free fatty acids (FFAs). Monoacylglyceride (MG). Diacylglyceride (DG). Glycerol (G).

provide such advantages over free enzymes [25].

Despite gaining stability and reusability, when enzymes are immobilized, additional factors start to play a critical role in the hydrolysis of waste FOGs. These include the interaction of reactants with the support, the interfacial area between the immiscible reactants, the accessibility of reactants to the active sites, the stability and activity of the anchored enzymes, and the potential effects of impurities, etc. [26]. These issues have been actively studied, and different approaches have been explored to overcome the resulting limitations. Some studies have been focused on developing mesoporous supports with large surface area or with tailored surface characteristics to improve reaction performance. Also, different immobilization methods have been developed to improve enzymes stability and activity [23]. Other studies have focused on the use of intensified technologies to increase compatibility and interaction between reactants. For instance, enhancing mixing and promoting the formation of stable emulsions. In this latter cases, techniques such as high-shear mixing, microwaves, and ultrasound have been proven to be effective in promoting contact between reactants and generating high hydrolysis rates of lipids, even in solvent-free systems [27].

Despite the recent advances in the hydrolysis of waste lipids, there is still a need for a better understanding of the enzymatic processes, immobilization techniques, supports characteristics, processing conditions, intensification methods, and reaction configurations. This in turn would enable to develop suitable technologies for industrial-scale production of FFAs from second generation feedstocks [28]. The aim of this study is to develop a comprehensive and critical review on the production of FFAs via hydrolysis of waste FOGs. The paper includes the description of different waste lipids suitable as oleochemical feedstocks, the available commercial process for their harnessing, their operating conditions, the developments on catalysts for hydrolysis, and the recent advances on intensified technologies to improve the process performance. Finally, some prospects and future directions regarding the reclaiming and valorization of waste lipids via industrial hydrolysis are discussed.

2. Waste lipids as oleochemical feedstocks

As a result of their availability, biodegradability, and renewable character, edible oils and fats have been traditionally used as primary feedstocks of the oleochemical biorefineries. Current production of vegetable oils is around 210 Mt/yr., and about 20% is used for biofuels and oleochemicals manufacture [11]. The main edible vegetable oils currently used for oleochemical applications are obtained from African palm, soybean, rapeseed, sunflower, palm kernel, cottonseed and coconut. These resources have been transformed in numerous derivatives for a variety of applications, and through different chemical pathways, some of which are described in Fig. 2 [2,29]. Nonetheless, the use of edible oils for biofuels and chemicals production involves many sustainability and ethical issues considering the water-energy-food security nexus of agricultural commodities [30], and the large life cycle impacts of agriculture, mainly in terms of land change use, fossil resources intensity, and biodiversity loss, among other indicators [28,31].

Alternatively, and following circular economy principles, waste FOGs can be used as second-generation feedstocks for the oleochemical industry. Despite the large content of impurities and humidity, waste FOGs are mainly composed of TAGs and FFAs that are suitable for further exploitation via hydrolysis, esterification, transesterification, etc. Recently waste FOGs availability has substantially increased as a result of a higher consumption of vegetable oils [11], and their prices have been lower than those of most virgin vegetable oils [32]. For instance, a well-known inevitable waste FOG is used cooking oil (UCO) which is discarded after food preparation. Recent studies indicate that global UCO generation is around 20–30% of the total production of vegetable oils (i.e. 42–63 Mt/yr) [11]. Likewise, only Malaysia that is the major global producer of palm Oil, generates around 60 Mt/yr of palm oil mill effluents (POMEs) which are agroindustrial waste FOGs that cause serious health and environmental impacts [5,33,34]. POMEs are lipidic residues from palm fruit sterilization and milling processes contained in wastewaters that have a chemical oxygen demand (COD) in between 15 and 100 g/L. These lipids cannot be collected by decantation because the low concentrations and because natural surfactants promote stable colloidal suspensions. Currently, ponding and digestions are

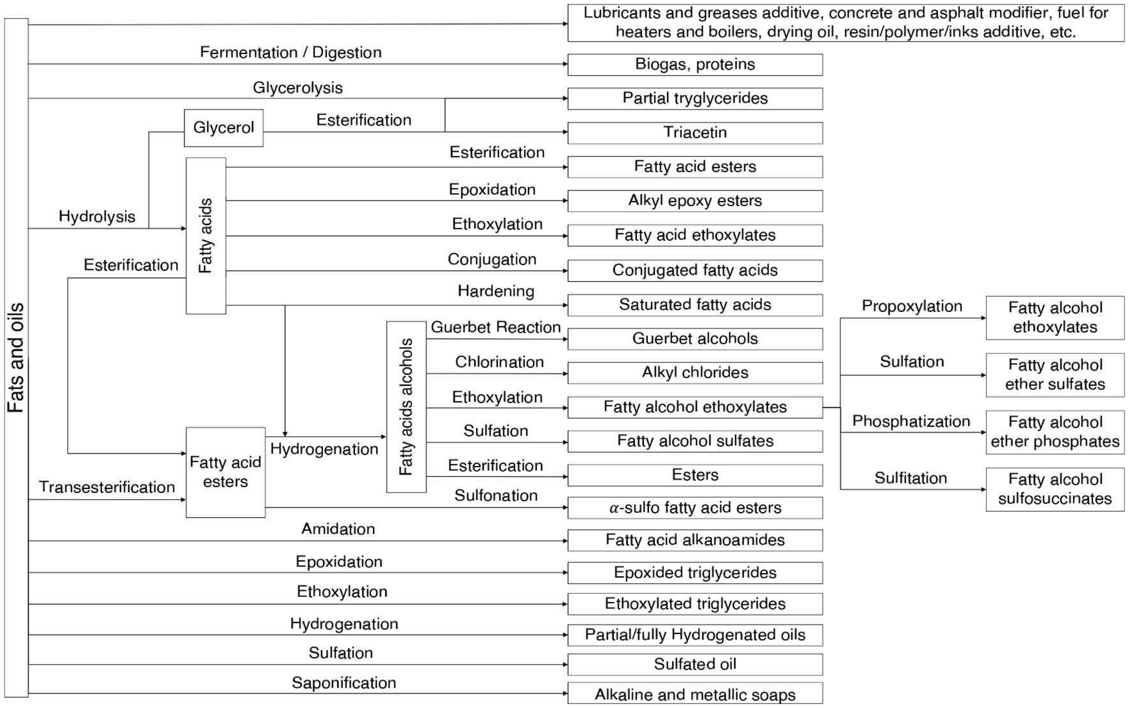

Fig. 2. Traditional oleochemicals processes and products (Adapted from [2] and [29]).

mainly used for POMEs abatement, but those technologies are unsatisfactory, and require high capital investment and maintenance costs [33]. Similarly, in the edible oils purification, large waste streams are generated during deacidification (i.e. deodorizer distillates and soaptstocks) and filtration (i.e. spent earths) [18]. Then, considering the global availability of waste FOGs, and assuming their exploitation can be accomplished, it would be possible to reduce a large fraction of the edible oils currently used in the oleochemical industry.

The global production of the different waste FOGs is very large, and it represents a major issue due to their extended mismanagement [9]. Typical waste FOGs correspond to inevitable food post-consumption residues and also to agroindustrial residual streams, including the above-mentioned UCOs, trap greases (i.e. brown grease), fats from animal rendering, extracted oils from food residues (e.g. fried food, expired mayonnaise, sauces and canned food), rancid oils, lipids from spent filtering and bleaching earths, soapstocks, mill effluents, and deodorization distillates, among others [9,35]. The first category corresponds to post-consumption food waste FOGs, which are mainly produced in urban centers, and they are generally disposed through the sewage systems or within the solid residues [36]. These practices trigger a cascade of environmental problems including sewage blockage, overfloodings, vectors proliferation, and excessive organic loading to waste waters and solid residues [9,13]. All this results in the damage of public and private infrastructure, and in the reduction of treatment plants and landfills effectiveness, thus causing extended pollution and ecosystems damages. Additionally, in some regions waste FOGs are illegally collected, bleached and redistributed as new oils, with the consequent impacts on public health [37]. As a result of all these problems, public agencies in different countries have deployed regulations to enforce the correct management of residual FOGs [38]. In the case of industrial waste FOGs, they are better regulated, but they still represent major costs because the loss of productivity and the mandatory actions for their treatment and disposal [9,39].

In order to mitigate most of the problems associated with waste FOGs management, exploitation for biofuels manufacture has been widely promoted, mainly for biodiesel and hydrotreated vegetable oil (HVO) production. Regulations such as the renewable energy directive (RED) of the European Union has boosted the use of waste-based biofuels by mean of subsidies or other type of benefits (e.g. double counting) [40]. Nevertheless, a maximum share has been established for such types of feedstocks [41], thus limiting the potential exploitation for the increasing volume of waste FOGs. In this regard, there is need to develop alternative harnessing routes to incorporate such residues within commodity or value-added chemicals [9]. Shall this be accomplished, this would help the oleochemical industry to incorporate circular economy models and to achieve a better sustainability performance.

A major challenge when employing waste FOGs as feedstock is related to their characteristic heterogeneity and the high content of impurities (e.g. humidity, hydrocarbons, polymers, polar compounds, food residues, etc.). Some characteristics of typical residual FOGs are listed in Table 1, and as observed, these residues exhibit higher acidity and moisture content than commercial edible oils. However, they still have a large fraction of TAGs containing value-added unsaturated fatty acid chains. For this reason, different alternatives other than biofuels have been reported for their industrial harnessing, these include the production for low-quality soap, fodder additive, bio-lubricants, asphalt binder, surfactants, biopolymers, biogas, etc. [18,32,42–46].

Sundry studies have indicated that the composition of waste FOGs is critical when establishing feasible exploitation routes. For instance, moisture and FFAs could affect reaction performance if a suitable catalyst is not properly selected. This is the case of processes that use alkaline catalysts (e.g. transesterification, dehydration) because the high content of FFAs can generate soaps and water that can trigger hydrolysis [30,68]. In the case of enzymatic processes (e.g. hydrolysis, esterification, transesterification), it has been observed that impurities in waste FOGs can inhibit the bio-catalyzed reactions [69,70]. As a consequence, most current approaches to harness waste FOGs involve intensive pre-treatment processes. This represents additional costs, the generation of secondary wastes, and the reduction of productivity. Alternatively, these problems could be overcome by developing resilient processes able to withstand impurities, or with minimally pre-treated waste FOGs [30]. Among the different valorization routes, the extraction of FFAs, their synthesis via hydrolysis of TAGs, and their further transformation is of great interest due to the high acidity of residual FOGs [18]. For instance, waste oils from fish processing have been used in the production of polyunsaturated fatty acids (PUFAs), particularly of omega-3- and omega-6-enriched products [50,71,72]. Also, fatty acids from hydrolyzed POMEs has been recovered and used in the production of biolubricants [34]. Similarly enzymatic hydrolysis of soybean deodorization distillate has been evaluated as a middle stage for the purification of Tocopherols presented in those residual streams [35]. However, several challenges need to be overcome in the extraction or the hydrolysis of residual FOGs to enable the industrialization at existing oleochemical biorefineries.

Table 1
Typical properties and specifications of common residual lipids streams.

Specification	Free Fatty acids content (%wt.)	Moisture (% wt.)	Iodine value (g I_2/ 100g)	Saponification value (mg KOH/g)	Refs.
Used cooking oil	< 6.0	<0.51	> 75	>193.2	[47,48]
Oil from POME*	7.8	2.36	51	147.3	[49]
Waste fish oil	0.11	—	>94	>182	[50,51]
Trap grease	31.0	—	—	—	[52]
Yellow grease (Rendering)	< 15.0	<1.29	52	198.4	[53–55]
Brown grease	> 25.0	<1.00	—	—	[56]
Tall Oil	45-55	—	150-180	150-170	[57]
Sludge palm oil (SPO)	23.2 – 49.3	< 1.50	52.9	206.25	[58,59]
Lipids from food waste	0.62	<51	83.7	—	[60]
Deodorizer distillates	< 63.0	<0.36	100 -127	165 - 175	[61,62]
Soapstocks (Different sources)	<10	< 50.0	<100	<50	[63–67]

* POME – Palm oil mill effluent.

3. Hydrolysis of waste lipids

3.1. Non-catalytic processes

Fatty acids production via hydrolysis of oils and fats is well-known since the early days of the industrial revolution [2,73], and one of the most commonly used technologies is the uncatalyzed Colgate-Emery process (Fig. 3) developed in the mid 1900's.

Highly energy-intensive processes such as the Colgate-Emery has been employed in the hydrolysis of waste FOGS, operating at high temperatures (200–300 °C) and pressures (50–60 bar) to maintain reactants in the liquid phase and to achieve conversions up to 98% [74]. In this process, fat pumped from storage tanks is fed to the bottom of a pressure column where is put in countercurrent contact with water, using a steam side inlet to provide the required heat duty for the process. Inside the column a sparge ring breaks the fat into small droplets that rise through the column to enhance two-phase contact. In the top part of the column, a decanting zone enables the separation between fatty acids and water, while in the bottoms another decanting stage facilitates the removal of sweet water (i.e., rich glycerol phase). The produced fatty acids are discharged from the top of the tower by mean of a

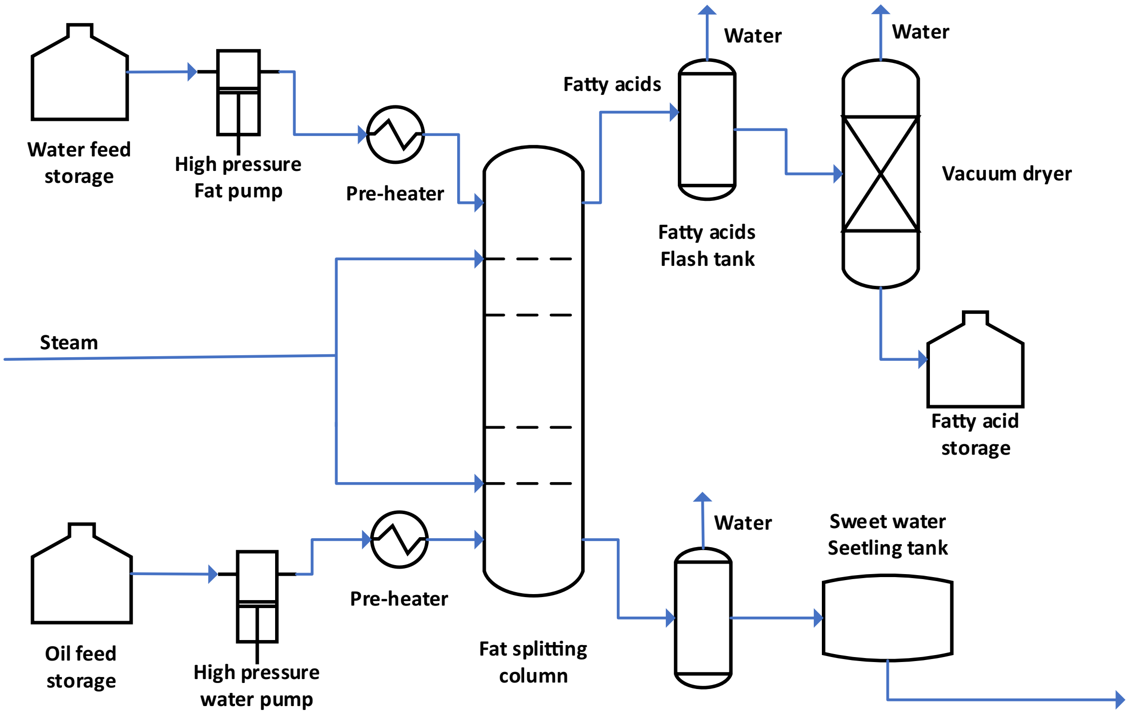

Fig. 3. Schematic process flow diagram of the Colgate-Emery process.

back-pressure control valve thus maintaining high pressures internally to avoid water vaporization [73]. For that same reason, some of the water is flashed in the tanks after top and bottoms streams are removed. Subsequent FFA drying is required and this is done by vacuum distillation.

An important limitation is such process is that, under such severe conditions, PUFAs tend to polymerize, and also there is formation of anhydrates which can also degrade into ketones and hydrocarbons [27]. Another drawback is the need for several separation steps including flash thanks, bleach reactor and distillation columns to purify fatty acids, glycerin concentrator, water de-aerator etc. Also a large amount of steam is required to obtain a high-quality product, thus increasing the overall production costs [3]. For this reason, the Colgate-Emery process has been recently revisited to enable harnessing of acid oleochemical waste streams in the production of biodiesel. In this route, the FFAs are obtained via complete hydrolysis of waste FOGs (e.g. trap greases, UCOs) and then converted into fatty acids methyl esters (FAMEs) via esterification. This process, known as hydro-esterification is currently used by Bioprax in Brazil in a 60 kton/yr. biodiesel plant. The process is carried out in a counter-current splitting column similar to that in Fig. 3, followed by the esterification step, employing a heterogeneous catalyst [75].

Alternatively, another pseudo catalyst-free pathway has been proposed including the use of supercritical CO_2 as pressurizing gas in water media. In this process solvation of CO_2 induces the formation of H_2CO_3, which also may play role as acid catalyst. However, CO_2 also tends to dissolve in the oil phase which also may have a negative effect in the kinetics due to the decrease in the FFA autocatalytic effect [74]. In general supercritical processes have demonstrated to overcome diffusion limitations and shortening reaction times during hydrolysis [35, 75], but subcritical conditions seems more suitable because the polarity of water and oil become similar owing to a shrink in dielectric constant of water. Under these conditions water acts as a proton acceptor resulting in a self-catalytic action, which is enhanced by a selective extraction of produced glycerol [74,76,77]. By using this approach, a 95% yield of FFAs was achieved after 2 h of reaction at 250 °C in the hydrolysis of UCOs and soybean soapstocks [75,77].

3.2. *Catalytic processes*

Catalytic hydrolysis is preferred over the non-catalytic routes because the process can be run under milder operating conditions, with less energy consumption, and with a reduced product degradation. Additionally, selectivity can be tuned for the controlled synthesis of the intermediate monoglycerides (MGs) and diglycerides (DGs) which can be valuable for some industrial applications [20]. In general, heterogeneous catalysts are favored over the homogenous ones to gain control over the selectivity and to enable an easier recovery, however there are some inherent drawbacks mainly related to deactivation, lower reaction rates, and mass transfer limitations that need to be overcome [3]. The manner in which such drawbacks are addressed in the hydrolysis of residual FOGs depends on the type of catalysts, either homogeneous or heterogeneous. Some details are described in the following sections. The specific case of enzymatic catalysts will be discussed in Section 4.

3.2.1. *Homogeneous catalysis*

A straightforward approach for the hydrolysis of low-quality triacylglycerides (i.e. waste FOGs) in the manufacture of FFAs is the use of homogeneous acid catalysts (e.g. sulfuric acid) as in the Twitchell process [74]. In this case, hydrolysis is run at the boiling point of water under a highly acidic aqueous media [78]. In some cases, soapstocks are also treated with sulfuric or hydrochloric acid in a so called splitting or acidification process, to recover FFAs and hydrolyze part of the still remaining TAGs [18]. However, this chemical route has several limitations such as equipment corrosion, side reactions, a high water intensity, a high reaction time, a massive industrial wastewater generation, and a lower hydrolysis degree (80–85%) [3,79]. In particular, lower rates of hydrolysis resulting in long reaction times (15 h) have been observed when this process is used to harness UCOs using sulfuric acid as catalyst [80].

Alternatively to the acid catalysts, alkaline hydrolysis of oils and fats has also been carried out, in particular with residual TAGs [59]. However, in this case, saponification reaction occurs (i.e. soap-making) and the product is the corresponding alkaline salt of the fatty acids [81]. Commonly, alkaline hydrolysis of oils and fats is carried out in aqueous or alcoholic media with the addition of potassium hydroxide [82,83]. Despite the process is well known, this route produces a large amount of hard water in the downstream process, and the reactive media exhibits a high increase in viscosity mainly at low temperature due to soaps and glycerol production [3]. This last characteristic reduces conversion and creates difficulties for transport phenomena and subsequent product separation. Another disadvantage is that the recovery of FFAs is carried out by means of an energy- and materials-intensive downstream purification involving soaps acidification. This is mainly carried out with strong homogeneous inorganic acids, thus generating the corresponding salts as residual stream (Fig. 4) [59,84]. Despite this approach has similar limitations to those of the acid hydrolysis of TAGs, the main advantage is that both reactions (i.e. saponification and acidification of soaps) are almost quantitative, thus waste FOGs conversion into FFAs is nearly a 100% [83].

Other homogenous processes for the hydrolysis of waste FOGs involve the use of free lipases as catalysts. While this has been widely studied, the process presents a great number of drawbacks mainly related to the instability and high costs of such bioderived molecules [19]. In general, denaturing and deactivation of enzymes caused by impurities of waste FOGs, difficulties in their recovery, and the high costs, all make enzymatic processes difficult to implement in the production of industrial chemical commodities. One of the firsts attempts to implement the enzymatic process for the production of FFAs at the industrial scale was done in the 1990s. In this process lipase from *Candida Rugosa* lipase (CRL) was used in the hydrolysis of beef tallow from rendering plants and linseed oil, in a facility that produced nearly 4 kt/yr. of FFAs. However, due to the high cost of the catalyst, the non-easy recovery, the observed inhibition, and the instability of the enzyme, the plant was decommissioned [85].

3.2.2. Heterogeneous catalysis

During the last decades, the interest for developing processes that use heterogeneous catalysts (e.g. immobilized lipases, acid solids and membranes, supported metals) for the hydrolysis of waste FOGs has increased. However, despite the process could be carried out under milder conditions facilitating catalyst recovery, most heterogeneous catalysts have demonstrated lower activity than homogeneous ones and also mass transfer limitations. Additionally, in some case the use of solid catalysts in hydrolysis have involved the use of organic solvents, making it a non-environmentally friendly approach [3]. On the other hand, the use of immobilized enzymes is still under continuous development considering the need for overcoming the reduction of activity of the anchored enzyme, and the need to mitigate enzyme leaching. Nevertheless, heterogenous catalysts seem to be a suitable alternative to overcome the limitations related to the traditional waste FOGs hydrolysis process, and they are superior over homogenous ones because they avoid equipment corrosion and easy separation of the products [86]. A summary of processing conditions during the catalytic hydrolysis of different waste FOGs is presented in Table 2.

3.3. Process limitations

The lack of compatibility between reactants and the resulting mass transfer limitations are major drawbacks during hydrolysis of waste FOGs. Under typical reaction conditions, TAGs and water are immiscible, and some studies have established that without good miscibility between water and oil the mass transfer between both phases is the limiting kinetic step during hydrolysis [28,74]. This is typically recognized in the sigmoidal behavior of the kinetic profiles where the rate of reaction is slow at the beginning and the end, but faster at intermediate stages [89]. This is explained by the changing nature of the reactive media and the surfactant action of intermediate glycerides. Initially, formation of DGs and MGs aid emulsifying the immiscible reactants, enhancing mass transfer and therefore the reaction rate [3]. However, once glycerol is formed, there is an opposite effect since it increases the size of the droplets of the dispersed phase, reducing contact area, and consequently affecting mass transfer and reaction rate [90]. These issues could be overcome by operating at high temperatures, however degradation of PUFAs and other side reactions can occur, and this is exacerbated when working with waste FOGs.

Likewise, the simplest way to reduce mass transfer limitations without adding a solvent to generate a single phase, or an emulsifying agent, is to increase the total free interfacial area by inducing turbulence

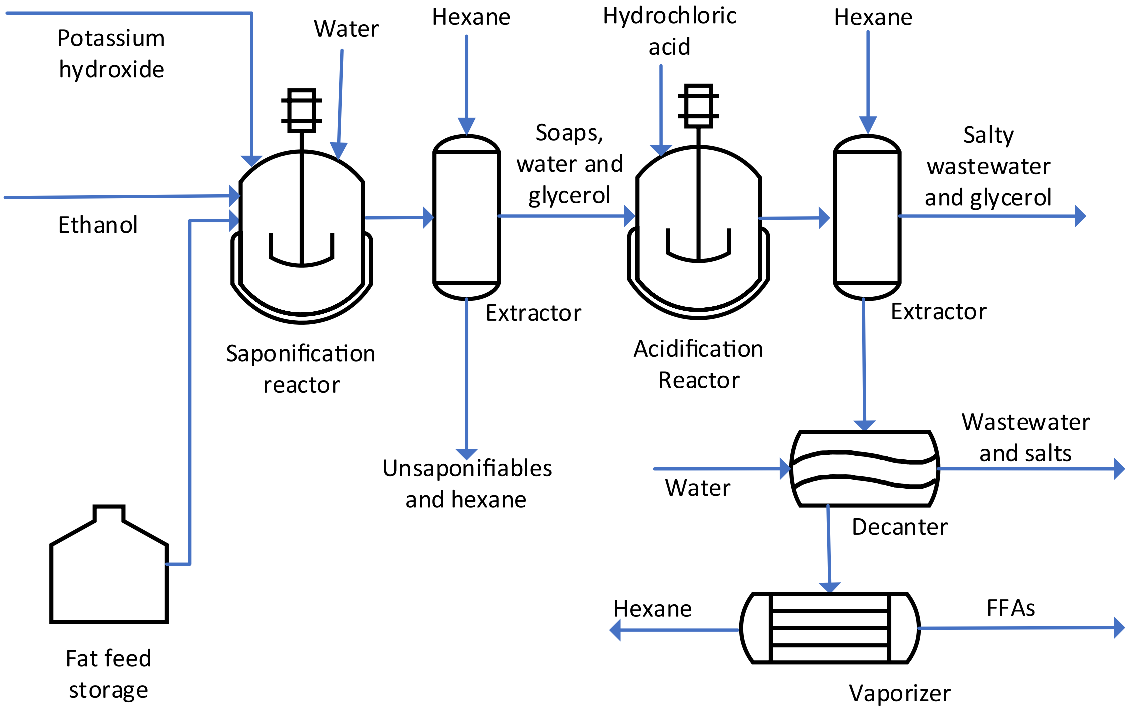

Fig. 4. Schematic process flow diagram of the saponification-acidification process.

Table 2
Processing conditions during the catalyzed hydrolysis of waste FOGs.

Waste FOG	Catalyst	Temperature (°C)	FFA yield (%)	Time (Minutes)	Remarks	Refs.
Waste fish oil	KOH - Sulfuric Acid		—	—	Alcoholysis of waste fish oil and further acid hydrolysis of FAMEs to obtain FFAs	[84]
Sludge palm oil (SPO)	KOH HCl	60 30	—	30 30	Direct saponification of SPO and furthers acid hydrolysis.	[59]
Used cooking oils (UCOs)	$CuSO_4$		60	120	Supercritical water system with $CuSO_4$ solution as catalyst and 1:30 molar oil to water ratio.	[74]
Soapstock	Sulfuric acid	90	>99	24	Acid hydrolysis of soapstock to obtain FFAs for Microwave Assisted Biodiesel Production.	[87]
Beef tallow	Ni or Pd supported on Alumina	290	>85	60	Production of stearic acid by *in-situ* hydrogenation of unsaturated FFAs	[79]
Used soybean oil	Solid Fe-Zn double-metal cyanide	190	83.2	12	Hydrophobic surfaces capable of adsorption and activation of TAGs molecules on active sites are responsible for high hydrolytic activities.	[3]
UCOs	Cu^{2+} from wastewater	225	170.0[a] (60% wt.)	—	Simultaneous hydrolysis of residual TAG and extraction of heavy metals in waste waters, reveals the feasibility of the process.	[88]
UCOs	Sulfuric acid	95	173.3[a] (61% wt.)	—	Acid hydrolysis (Twitchell process) indicates the low hydrolysis rate and the necessity of emulsifiers to obtain relatively high acid values.	[80]

[a] Acid value in mg KOH/ g oil and weight percent as oleic acid (% wt.).

via mechanical stirring [27]. However, operating under high stirring rates can derive in enzymes denaturing due to high shear stresses [24,91]. Alternatively, turbulence can be induced by mean of Intensified techniques such as cavitation, ultrasound and microwaves. These can help increasing interfacial area and generating local hot spots for short times, boosting rates of reaction but without involving shearing stresses. These particular characteristics make the intensified technologies attractive for industrial implementation [28].

On the other hand, the chemical equilibrium constant of TAGs hydrolysis is around 0.45 at 225 °C, which limits the maximum reaction conversion below 90% [91,92]. This can be shifted by using a molar excess of water ($>$ 3:1) [32,93] or by selective removal of glycerol or fatty acid from the reaction medium [28]. As there is a very large molecular weight difference between reactants ($TAG/H_2O \approx 44–50$), the process is generally run under high excess of water. However, large water loading can also increase the energy consumption of the whole process, mainly in the downstream separation. Also, additional mass transfer limitations may appear due to the low miscibility of reactants [20,94]. Beside, in the case of enzymatic hydrolysis, water plays a key role in the structural configuration of the enzymes and consequently in their activity. Despite a water layer formed by hydrogen bonds is important to retain the three-dimensional structure of the enzyme, an excess of water can derive in multilayer adsorption with formation of a thick aqueous film. This film results in a poor solubility of immiscible substrates (i.e. TAGs), a reduction of accessibility for non-polar and large molecules to the active sites, and also making the enzymes more susceptible to denaturation [20]. Also, in the enzymatic processes, the amount of water determines the required amount of buffer in the reactive medium [95] as buffer components are dissolved in the aqueous phase. Regarding the temperature dependence of the chemical equilibrium, there is a minor effect as the TAGs hydrolysis has a low heat of reaction [96].

Additional factors to consider during the hydrolysis of residual FOGs is their heterogeneity and its effect on the reaction performance. For instance, the melting points of non-edible and residual FOGS are usually higher than most typical edible oils ($>$ 35 °C), as they contain a larger fraction of saturated TAGs. This becomes a limiting factor for the use of bioderived catalysts which are active and stable under milder temperatures (25–40 °C) [19,70]. Also, solid particles, polymers, gums, phospholipids, sterols and tocopherols typically present in waste FOGs can block the porous matrix of heterogeneous catalysts and supports affecting mass transfer and rates of reaction. In this case, a suitable filtration and degumming might be required to reduce catalysts deactivation. Additionally, polar compounds and impurities in waste FOGs can generate problematic volatiles during processing, and additional organic loading for the downstream wastewater treatment. Finally, there are particular issues with the use of waste feedstocks associated with their sensory properties. In general, waste FOGs are yellowish or dark-brown materials, and their odor could be characteristic of food residues. As a result, there is need for energy- and materials-intensive pre-treatment processes for the waste FOGs or to carry out refining processes for the oleochemical derivatives in order to fulfill commercial products specifications (e.g. light color, odorless products). Thereby, effectiveness of such purification steps would determine the final application and the value of the waste-based oleochemicals, thus affecting the economic feasibility of the corresponding harnessing approaches.

4. Enzymatic hydrolysis of waste FOGs

The use of enzymes as bio-derived catalysts in the oleochemical industry has been widely studied because of their well-known key role in different metabolic processes and reactions. As previously mentioned, some of the most important industrial enzymes are lipases, which are primarily used for the catalytic hydrolysis of TAGs. They are produced by living organisms to aid the digestion, transporting, and processing of dietary lipids and TAGs [97]. The industrial interest on lipases as bio-based catalysts for waste FOGs hydrolysis relies on their high catalytic activity near ambient conditions, high selectivity, and a higher tolerance to impurities (i.e. FFAs and water) [18,98,99].

Nonetheless, and in spite of the benefits, lipases are usually expensive, difficult to recover, and are sensitive to reaction conditions such as temperature, pH, ionic strength, and impurities, thus limiting their industrial implementation [5,19]. In particular, one of the most challenging drawbacks in the enzymatic hydrolysis of waste FOGs is their content of polar impurities such as the organics generated after fats and oils oxidation (e.g. ketones, aldehyde, alcohols), or the phosphatides, sulfur- and nitrogen-containing compounds [16,17,78]. These molecules may be potential enzymes inhibitors since they could compete with TAGs and water for the active sites during hydrolysis, or they can promote desorption and leaching of anchored enzymes [69]. Beside, polarity of such impurities can generate a change in the morphology of enzymes, thus affecting their activity and selectivity. For instance, it has been verified that phosphatides present in waste and nonedible oils inhibit enzymatic reactions [100], and that the process can be improved by reducing waste FOGs to enzyme ratio [30].

In order to exploit enzymes advantages in the hydrolysis of waste FOGs, and to overcome the above-mentioned drawbacks, different techniques have been developed for the synthesis, isolation, purification, and immobilization of the biobased catalysts. Such techniques have enabled enhancement of the enzymatic performance and to conduct continuous processes in a variety of industries, including food, dairy, detergents, and pharmaceutical [101,102]. A successful example of these techniques is the enzymes immobilization on suitable supports such as ion exchange resins, inorganic aluminosilicates, activated carbons, etc. As a result of such improvement, immobilized enzyme Novozyme® 435 (i.e. lipase B from *Candida antarctica* on a resin Lewatit VP OC 1600; Novozymes) is currently the most widely employed biocatalyst for research and industrial synthesis [103]. Nonetheless, beside immobilization, different approaches have been explored to overcome the challenges of working with waste feedstocks. In this regard, and to enable their effective use as a catalyst in the treatment of residual lipids, different studies have focused on identifying alternative lipase sources, improving surface characteristics and porosity of the supports, developing more effective anchoring methods, carrying out enzyme modifications, engineering novel reaction media, and doing enzyme engineering, among others.

4.1. Immobilization of lipases in the hydrolysis of waste FOGs

Immobilization is the confinement of an enzyme to a phase (matrix/support) different from the reactive media [23]. It is done to overcome inherent limitations related to enzymatic reactions, providing more resilience to reaction conditions, and enabling a simpler recovery and reuse [5,104]. Some typical methods for enzymes immobilization are described in Fig. 5, which include adsorption, crosslinking, covalent bonding, entrapment, and encapsulation. Among these, the most widely used at the industrial scale is the physical adsorption because it is a simple, economic, and fast technique. Adsorption exploits the physical interactions generated between the carrier and enzyme, which occurs through Van der Waals forces, ionic interactions, and hydrogen bonding [102]. As the physical Interactions between enzyme and carrier are weak, it is a suitable technique to prevent enzymes distortion and to avoid a loss of activity [23]. Nevertheless, this weak interaction also means that absorbed enzymes are also prone to desorption and leaching.

Once immobilized, enzymes performance depends on several factors such as the employed immobilization technique [101], the physical and chemical properties of enzyme and carrier, enzyme conformation and loading, the nature of the immobilization and reactive media, substrate diffusion, and water activity [5,99,105]. In the special case of lipases an additional factor plays a role, namely the interfacial activation. In some lipases, there is a peptide chain called lid that, in contact with non-polar solvents or hydrophobic surfaces, can unblock and expose a hydrophobic pocket, which is the active site for hydrolysis. This behavior, schematically described in Fig. 6, has been exploited and typically used for the immobilization of lipases on hydrophobic carriers (e.g. activated carbon) [106].

In the specific case of hydrolysis of TAGs, there is production of FFAs, DGs, and MGs, within a water-rich media. Under these conditions, hydrolysis products exhibit a detergent action that can favor enzyme leaching [107]. This indicates that the simple physical adsorption cannot be suitable for the effective immobilization of lipases that are intended for the hydrolysis of waste FOGs. Alternatively, mixed immobilization techniques can be used to overcome the leaching issues [103,106]. Frequently, the approach used to enhance lipases stability is the combined adsorption and cross-linking with glutaraldehyde, a bifunctional agent which remains as a powerful crosslinker molecule. This strategy takes advantage of the reactivity of the carbonyl group in the glutaraldehyde with the amino groups in the enzyme molecule (Fig. 7). On the one hand, physical adsorption allows the linkage of the enzymes

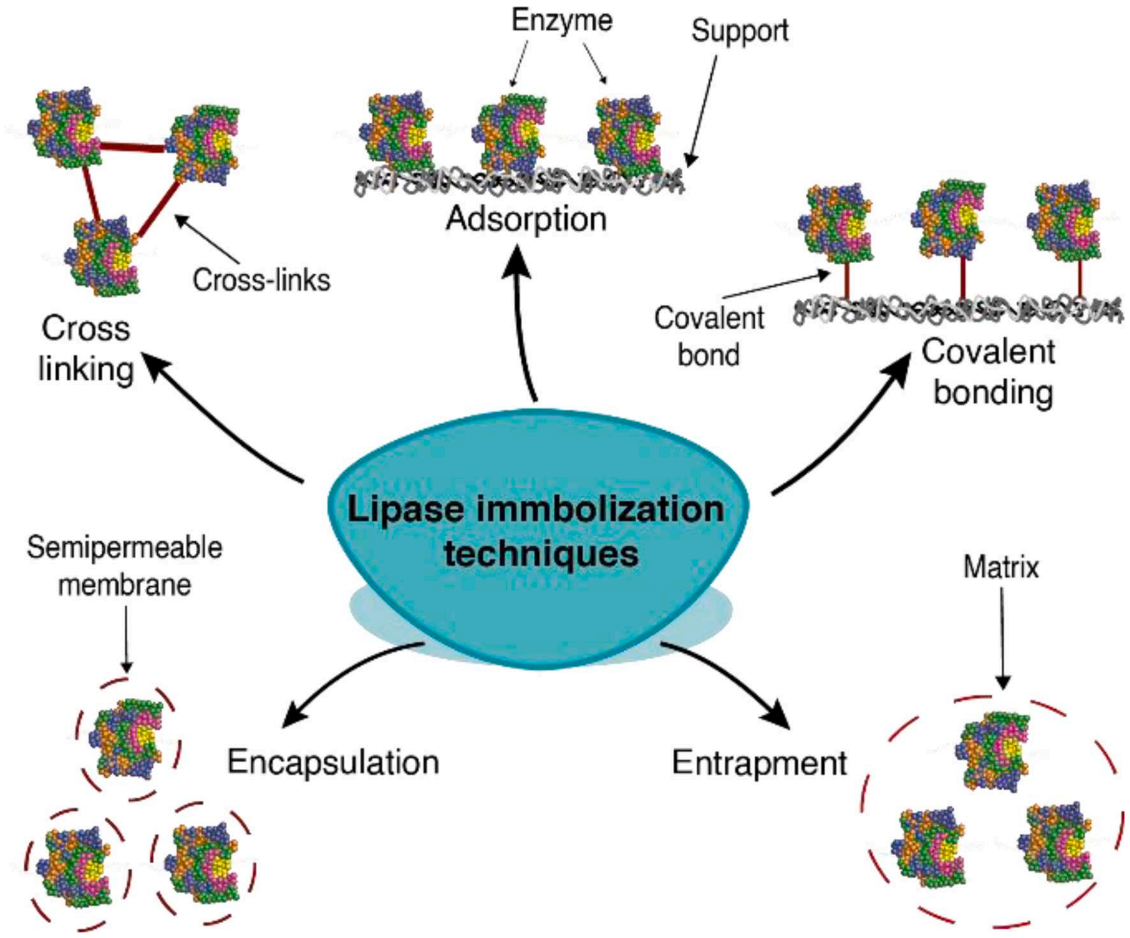

Fig. 5. Common immobilization techniques for enzymes.

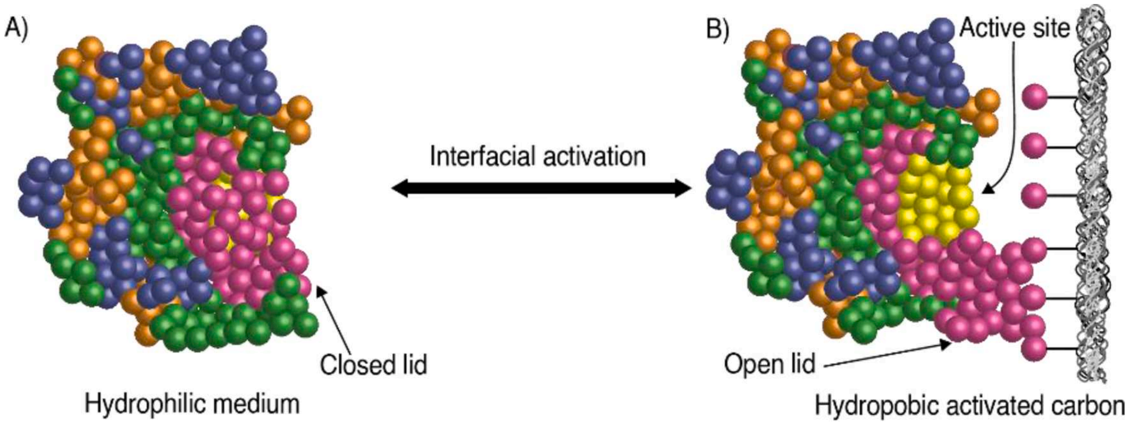

Fig. 6. Interfacial activation of lipases on hydrophobic supports. (A) Closed-form of lipase. (B) Open form of lipase.

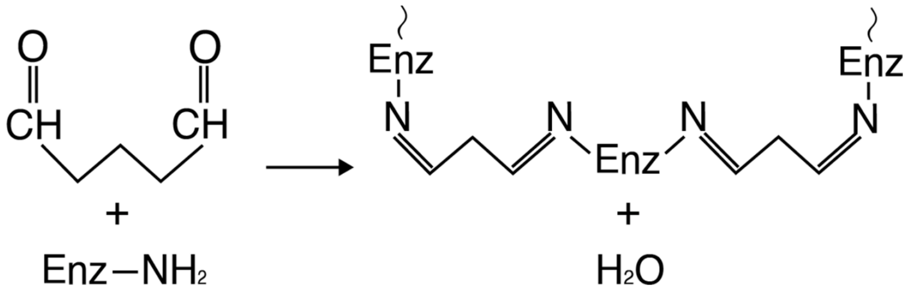

Fig. 7. Reaction schema of glutaraldehyde with protein molecules in acidic or neutral media.

to the carrier, and on the other hand, crosslinking enhances the stability producing enzyme aggregates inside the pores of the carrier, increasing the contact area between the enzymes and the bindings of the carrier [106,108].

Depending on the conditions of the media, glutaraldehyde behaves in different ways. For instance, in basic media glutaraldehyde tend to polymerize and the interaction between glutaraldehyde and the enzyme becomes weaker. Some studies have also shown that the amount of glutaraldehyde in the immobilization is a key step to ensure the activation of the amino groups in the enzyme without activity loss. Then, in order to enhance the crosslinking effect, to reduce glutaraldehyde loadings, to avoid enzyme inhibition, and to reduce costs, previously aminated supports are preferred for immobilization [106]. This occurs because the carbonyl groups show affinity toward nitrogen in amine groups, thus facilitating bonding between proteins and hydrophobic functionalized or non-functionalized carries [105]. Once the lipase is immobilized on the hydrophobic support (with or without assisted crosslinking) some factors could affect the reaction performance, such as temperature, pH, substrate, lipase source, and the supports. Despite most studies of enzymatic hydrolysis of FOGs using immobilized enzymes are performed with commercial lipases (e.g. Novozymes 435), some of them have focused in obtaining new biocatalysts as shown in the Table 3.

4.1.1. Lipase sources

Enzymes characteristics and performance are highly dependent of the source, and can sharply change even when obtained from a particular type of microorganisms [21]. In general, enzymes correspond to a consortium of macromolecules with catalytic action over specific bonds and reactions. Then, considering the heterogeneity of waste FOGs, the

Table 3
Immobilization studies for hydrolysis of FOGs.

Lipase	Carrier	Immobilization method	Optimum pH	Optimum Temperature (°C)	Remarks	Refs.
Candida Rugosa Lipase (CLR)	Sol–gel/calcium alginate beads	Entrapment	6.8	37	Evaluating the influence of ratio of sol-gel to alginate in the immobilized activity	[109]
Rhizomucor miehei intracellular lipase	Polyurethane foams / biomass support particles	Cell immobilization on biomass support particles	4.5	40	Cell aggregates inside porous carriers enhance the intracellular lipase activity	[19]
CLR	Polyvinyl alcohol (PVA) -alginate-sulfate beads	Covalent attachment	7.5	—	Covalent attachment to PVA beads conferees to the enzyme a rigid structure in the presence of UCOs.	[5]
CLR	Diaion HP-20	Adsorption	5.0	30	Changes in pH have more influence in enzyme conformation than hydrophobic interactions.	[99]
CRL	Zeolite	Adsorption	—	—	Adsorption of the substrates to the carrier surface can result in enzyme inhibition easily avoid working in pressurized systems.	[35]
Pseudomonas gessardii	Mesoporous activated carbon	Adsorption – Covalent interaction	3.5	35	Surface functionalization of activated carbons with amino and aldehyde groups enhances the covalent interaction after physical adsorption.	[105]

presence of potential inhibitors, and the need to operate under slightly high temperatures to ensure complete melting, a suitable selection of the lipase source is paramount to ensure high yields and stability during hydrolysis of residual lipids [110].

The specificity of an enzyme to a substrate is a captivating property that is currently exploited in the food and pharmaceutical industry [21,23]. Nevertheless, for the hydrolysis of waste TAGs, non-specific lipases are rather preferred than the specific ones in order to handle the heterogeneous fatty acid chain compositions of the feedstocks [18,111]. It has been found that during enzymatic hydrolysis, specific lipases would not reach high yields, thus producing a considerable concentration of MGs and DGs in the product. An exception occurs with the 1,3-specific lipases which enable to reach almost complete conversion to FFAs. In this case, the acyl migration relocates the fatty acids to position 1, making it more accessible to the lipases for further hydrolysis. However, these phenomena on such specific enzymes require longer reaction times [104]. In comparison, the use of lipases specific to long-chain fatty acids combined with non-specific one (i.e. combi-lipase), could enhance the hydrolysis of waste FOGs and the yield to FFAs [103].

Lipase from *Aspergillus niger* (*A. niger*), a widely known non-specific lipase, has demonstrated to have good hydrolytic activity over waste FOGs and good stability at low temperatures (-1 °C and 10 °C). However, while *A. Nigger* lipase has good activity with FFAs of medium (C12:0) and long chains (C18:0), it does not catalyze reactions with palmitic acid and other saturated chains [24]. In contrast, lipases from *Rhizomucor miehei* has demonstrated high conversions of TAGs (>85%), but with low relative yields of FFAs, mainly as a consequence of the waste oil impurities [19]. Similarly, the free form of lipase from *Geotrichum candidum* (GCL-I) can effectively hydrolyze UCOs in short reaction times (~40 min). This can be explained by GCL-I preference for the long-saturated fatty acid chains, which in general are in high concentrations in UCOs [30].

A widely studied and very effective lipase for waste FOGs hydrolysis is *Candida Antarctica Lipase B* (CALB). It is a non-specific lipase with high lipolytic activity and with high resilience to polar inhibitors (e.g. methanol, ethanol) [95,103]. Similarly, it was verified that *Penicillium chrysogenum* lipase displayed suitable activity for hydrolysis of TAGs with C-18 unsaturated fatty acid chains in UCOs, even under high inhibitors concentration in the feedstock [112]. Similarly, some authors have also established that *Candida rugosa lipase* (CRL) could be used successfully in the hydrolysis of wastewater rich in FOGs for further anaerobic treatment and animal fats (e.g. non edible lard pork and beef tallow), since CRL exhibits some selectivity to cis unsaturated FFAs in position 9 [70,109]. However, despite the high lipolytic activity reported for CRL, it has a poor activity when employed in UCOs, reaching complete conversion to FFAs only after very long reaction cycles [113]. Even though it was believed that CRL could be employed in the purification and extraction of PUFAs from waste fish oil, hydrolysis conversion below 24% was achieved with free CRL as biocatalyst [50]. In the same sense, halotolerant lipases (i.e. lipases tolerant to high salinity media) from *Bacillus sp.* VITL8 has been used in the pretreatment of bakery, dairy and poultry effluents, requiring short reaction times (i.e. 8 h) with a relative high production of FFAs in 8 h [114].

More recently, some authors have explored lipases from plants for the hydrolysis of UCOs, identifying that in some cases, they are very active even without the addition of co-solvents and cheaper than microbial ones. In some cases, those lipases have also shown resistance to the, and a 1,3-selective behavior [115]. Following the same direction, microbial lipases from marine microorganisms have also been tested for hydrolysis of waste FOGs, owing to the extreme conditions of marine life that give as result enzymes with boosted activity and stability, suitable for the biotechnology industry [116].

4.1.2. Supports

Despite the effectiveness of some enzymes, their major limitation is the high cost; in general it would be required up to 100 cycles of re-use to ensure profitability at the industrial scale [100]. This have called the attention on developing suitable supports to facilitate recovery and reuse, ensuring activity preservation. However, immobilization on such supports further increases the costs of enzymes. For instance, it has been determined that nearly 47% of the total cost of immobilized enzymes is attributed to the cost of the support (e.g. Novozyme 435) [117]. Thus, selection of suitable supports must be accomplished considering technical and economic considerations.

The properties and physicochemical characteristics of a support are of upmost importance in the performance of an immobilized enzyme [23]. An ideal matrix must have specific features like inertness, physical strength, stability, regenerability, nonspecific adsorption, ability to increase enzyme specificity/activity and reduce product inhibition, and resistance to microbial contamination [118]. Highly hydrophobic mesoporous supports may improve the yield in hydrolysis reactions by reducing the accumulation of glycerol and water in the surface when using lipases as catalysts as shown in the support used in Novozyme 435 [97,104,119]. Also, it has been reported that hydrophobic supports improve the diffusion of the substrates and products [108]. As the immobilization and reaction temperature is generally low for the different lipases (i.e. around 37 °C), thermal resistance is not a main factor when selecting the supporting materials [120]. In contrast, other selection criteria based upon the properties of the support and its interaction with the lipases and reactive media must be considered. Some of the most important selection criteria for immobilization supports are summarized in Table 4.

Hydrophobic interactions have been widely studied when selecting and characterizing supports for lipase immobilization, because reversible interfacial activation dictates stability and reusability [122]. In the same way, since hydrophobicity of the carrier influences lipase activity, studies have shown that it can be increased by pretreatment with ethanol [123]. Some enzymes have also shown specificity for some hydrophobic supports, enabling to separate and differentiate lipases [122]. Also, enzyme activity is highly dependent on the pore size of the carrier; at least five times the diameter of a molecule is required in the pore size to avoid diffusional issues, but larger pores than 100 nm result in poor enzyme stability [124].

Among the different assessed supports, activated carbons (AC) have shown suitable performances in the hydrolysis of waste TAGs [105]. These materials have exhibited good structural properties, high substrate stability, and low costs [125]. Additionally, it has been verified that one of the most promising enzymes for waste FOGs hydrolysis, *Candida Antarctica*, has less textural and conformational changes on mesoporous carbon materials [126]. In general, the interactions of lipases with AC are weak at high temperatures, which leads to a shrink in the adsorption capacity [127]. Comparatively, lipase adsorption onto AC results optimal at temperatures in between 30 and 40 °C, enhancing stability at conditions waste FOGs hydrolysis, and enabling to operate in wider range in comparison with free lipases [128]. Likewise, a similar study on immobilized *Pseudomonas gessardii* identified that mesoporous ACs are very effective supports for hydrolytic systems owing to their high substrate affinity, mechanical stability, and the enhanced accessibility for large TAGs [129]. Despite the promising characteristics of ACs as enzymes supports, further studies are required because potential adsorption of impurities from waste FOGs can drive to pores clogging or active sites deactivation. These phenomena could be mitigated by the use well defined mesoporous or macroporous structures [126,129] using suitable surface activation methods to enable effective enzymes anchoring with reduced affinity for organic compounds.

4.2. Variables that influence enzymatic hydrolysis of waste FOGs

4.2.1. Temperature effect

Temperature plays a significant role in enzymatic reactions since it is tightly related to the solubility, the fluidity of the reactive media, and to the viscosity of the substrates; all affecting mobility of reactants [91]. In

Table 4
Selection criteria for enzymes supports in enzymatic hydrolysis of waste FOGs.

Criteria	Support	Remarks	Refs.
Form (Morphology)	Polystyrene-divinylbenzene	High immobilized hydrolytic activities due to large pore size and high support surface.	[111]
Interactions	Polyvinylidene fluoride membranes	Lipases tend to exhibit high hydrolytic activities in supports with high hydrophobicity.	[121]
	Polyurethane	Mechanical interactions allow to obtain better stability than electrostatic ones in whole cell immobilization.	[19]
	Polyvinyl alcohol -alginate-sulfate beads	Interactions enzyme-substrate enhances the stability and the conformation of the enzyme, allowing to work with FOGs in wide ranges of pH and Temperature.	[5]
Reusability	Polyvinylidene fluoride membranes	Immobilized enzymes show an improved stability in different pH ranges and temperature storages than their free counterparts.	[121]
	Polystyrene-divinylbenzene beads (Diaion HP-20)	Diaon beads exhibited good reusability in the hydrolysis of UCOs in pack-bed reactors owing to pH stability.	[99]
	Mesoporous activated carbons (MAC)	Stability of MAC conferred to the lipase improves storage and number of recycles.	[105]

the other hand, energy of activation of physical bonding is lower than that of chemical ones, so the strength and rigidity of anchoring bonds of the enzymes are also affected by temperature which can provoke enzyme desorption. Finally, the internal bonds of enzymes are sensitive to high temperatures require more thermal energy to be in a denatured form; this phenomena has been verified using CLR supported in polystyrene-divinylbenzene for the hydrolysis of UCOs [99]. All these elements indicate that the immobilization process and hydrolytic reaction must be carried out under specific and optimal temperatures for a suitable trade-off between anchoring effectiveness and enzymes activity and stability.

In the case of hydrolysis of residual FOGs, Immobilized lipase of *Rizopus oryzae* supported on alginate beads permitted conversions above 90% at 40 °C in the enzymatic hydrolysis of POMEs [91]. Likewise, immobilized CLR has been tested in the hydrolysis of UCOs, and it was found that thermal stability can be affected by the immobilization process, conferring a rigid and stable enzyme conformation. This stabilization enables to operate under higher temperatures and to work with different waste FOGs [5]. Similarly, a study on the hydrolysis of waste FOGs using *Rhizopus japonicus* [21], indicated that the optimum temperature narrowly depends of two factors (i) the influence of temperature in the kinetics (ii) the rate of denaturation due to changes in the structural conformation of the lipase. Both factors determine a unique optimum temperature for each lipase and substrate used (i.e. type of waste FOG). This same behavior was observed using CRL in the enzymatic hydrolysis of soybean deodorization distillates, where temperature changes had a significant impact in the nature of reaction media [34]. The same enzyme (CLR) in its free form was also tested in the hydrolysis of acid oils but it exhibited reduced stability. In this case the optimal operating temperature of the free enzyme was 30 °C [130] which is a major limitation for the hydrolysis of FOGs when considering their higher melting points; in this case, lipase immobilization will be necessary to enhance temperature stability.

4.2.2. *pH effect*

pH is a main variable affecting the immobilization process and also the hydrolysis reaction mainly because physical binding of enzymes is affected by surface electric charges, and these are determined by the pH of the surrounding media. Also, the morphology of the enzymes is highly dependent on pH because protonation of some chains of amino acids occurs near the isoelectric point [78]. At this condition, enzymes are more stable and active, and in the case of lipases, they are usually positively charged [127]. For this reason, pH tuning and pH memory are exploited variables and characteristics in most lipase immobilization processes, and they mainly depend on the immobilization method and the interactions between the enzyme and the support [5]. In the other hand, once immobilized, it has been observed that the hydrolytic activity of enzymes is reduced at high alkaline or acid pH. This has been explained as an affinity reduction between the enzyme and the hydrophobic support that affects adsorption effectiveness [99]. For instance, in the case of *Rhizopus japonicus* lipase, it exhibited an optimal pH of 7 in the hydrolysis of a waste fraction from the production of hydrogenated fat, but it was verified that active sites of lipase are highly affected by pH, as well as the diffusion of the substrates trough the enzyme [21]. This reaffirms that, in their immobilized form, a minor change of pH can derive in a substantial activity drop (i.e. ~ 50%) of lipases [20]. Thereby, the control of pH at an optimal condition has been normally carried out by using buffer solutions during reaction. Nonetheless, this increases operating costs and generates difficulties during downstream recovery of glycerol from the aqueous effluent from hydrolysis.

Alternatively, pH tolerant lipases have been increasingly studied, particularly from novel sources (e.g. marine, fungi, intracellular). An interesting feature exhibited by fungi lipase is that those organisms produce isoform lipases; in the case of immobilized *Rhizomucor miehei* lipase used in the hydrolysis of beef tallow and poultry fat it has shown maximum activity between 6 and 8 pH range, while its free form only present high activity at pH 7 [19]. Similarly, immobilized CLR on polyvinyl alcohol-alginate-sulfate beads used in the hydrolysis of UCOs, have shown no changes in conversion when operating in a pH range of 7 to 8. While activity was maintained, the observed drop of FFAs production suggested that minor pH changes had an effect on the selectivity of the biocatalyst due to the change of interactions between the enzyme and the support [5]. Alternatively, pH stability has been boosted by using intracellular lipases in the hydrolysis of FOGs, because the intrinsic cell permeability enables to balance the pH change caused by the released FFAs [131]. Owing to their nature, marine lipases such as *Pseudomonas otitidis* have shown good pH stability in the hydrolysis of cooked sunflower oil, maintaining 72–83% of the activity within 5.0–6.0 pH range, 90% at neutral pH, and 85–55% in the pH range of 8–9.5 [116].

Despite the many studies verifying that pH is a key parameter during hydrolysis of waste FOGs, a promising study revealed that immobilized TLL can be used under uncontrolled pH medium, achieving nearly 100% conversion after 24 h [95]. These results open new opportunities to work in salt-free systems (i.e. unbuffered solutions) with acid-tolerant enzymes, thus facilitating downstream purification of glycerol, and making the process more sustainable.

4.2.3. *Reaction media and enzyme loading effect*

The substrate and solvent (if required) of the hydrolysis reactive media are normally different to that of the immobilization process. However, it has indeed an effect on the stability, leaching and denaturing of the anchored enzyme. Thus, suitable techniques must be developed to ensure that the anchored enzymes withstand reactive media and the corresponding operating conditions. In the case of hydrolysis using lipases, water play the role of substrate as lipases binds to the TG molecule in the water-oil interface. For this reason, several studies in the hydrolysis of waste FOGs are focused in identifying a suitable water to oil ratio because interfacial area depends of the amount of water [113]. Nonetheless, excessively high water or solvent loadings might not conciliate well with the features of the enzyme, leading to enzyme leaching from the solid support with the consequent decrease of activity [132]. Also, in the case of polar polymeric supports, water can swell the pores covering the actives sites. In this case there is not only a miscibility barrier for the waste FOG, but also a reduction in the activity of lipases because the hydrophobic interactions with the lipids are broken under the high ionic strength of the surrounding aqueous medium [122]. In the hydrolysis of UCO with CRL it has been observed that high water loadings induces a negative effect in the enzymatic activity, and this can be explained as a dilution and folding effect of the lipase due to the hydrophilic media [32]. The same effect has been verified when using CRL in the hydrolysis of deodorization distillates, where high water contents triggers phase splitting thus generating a sudden decrease in the degree of hydrolysis [35]. Usually the aqueous phase contains a buffer in order to control the pH, but a recent study suggested that buffered can contaminate the produced glycerol making more difficult its purification [95]. In order to mitigate the effect of substrates and/or solvents, immobilization of lipases for waste FOGs hydrolysis is commonly carried out over hydrophobic supports.

The presence of other additives in the hydrolysis of residual oils has been studied. In the case of organic solvents such as n-hexane, isooctane and cyclohexane, they have demonstrated negative performance when using *Tritirachium oryzae W5H* lipase in the hydrolysis of waste corn oil [133]. On the other hand, metal ions may have a positive effect in the stability of the enzyme in the hydrolysis of waste FOGs, a recently study suggests that *Pseudomonas otitidis* lipase is a metal-activated enzyme. In this case, ions join to the enzyme and change their conformation improving activity. Nevertheless, an opposite effect was observed in the presence of transition metals which had an inhibitory role [116]. The same study assessed the influence of surfactants in the reactive medium, and the results indicated that anionic surfactants (e.g. sodium dodecyl sulphate) had an inhibitory effect due to the negative charge.

Another significant parameter which has an effect on the enzymatic hydrolysis of waste FOGs is the enzyme loading. Large loadings are generally used to counterweight low activities, and several studies have stated that by modifying enzyme loading, up to 50% higher conversions can be achieved [133]. The same behavior has been observed when using CLR in the hydrolysis of UCOs however, there was an optimal loading because it also affects the hydrophobic interactions with the support. This study has also pointed out that, under suitable carrier and immobilization conditions, a lower amount of immobilized lipase was required to obtain the same activity in comparison with the use of the free lipase [5]. One of the issues of using excessive enzymes loading is that it promotes their agglomeration hindering the access of the substrates to the active site [115]. This effect has been verified in the hydrolysis of acid oil using free CLR; in this case high lipase loadings (>100 Units/g-mixture) have no positive effect in the hydrolysis degree due to the formation of inactive lipase-lipase aggregates [130]. A summary of the operating conditions during the enzymatic hydrolysis of waste FOGs in most recent studies is presented in Table 5.

Some of the above mentioned enzymes have reached commercial status and they have been used in a variety of processes [103]. In the case of the free and non-specific lipase used in Novozyme 435 (i.e. CALB), it has exhibited acceptable yields in the hydrolysis of FOGs [27]. Similarly, the 1,3 regioselective commercial compeer *Themomices lanuginosus* lipase (TLL) used in Lipozyme TL IM has exhibited good activity but under lower reaction rates [132]. This same enzyme in its free form has shown a good performance in the hydrolysis of soapstocks and olive pomace oil, and the results suggest that the same behavior and method for monitoring the reaction could be used with other substrates [136]. However, in both cases, the commercial immobilized versions seem to be not appropriate for hydrolysis reactions because the supports are of partial hydrophilic nature. As previously mentioned, it has been established that highly hydrophobic supports are preferred for hydrolysis in order to promote interfacial activation, and to avoid inhibition by surface adsorption of glycerol or water [103,104].

4.3. Enzymatic catalysts reusability

Enzymatic process feasibility is tightly related with a suitable technical and cost-effective performance. Studies attempting to recycle free lipases for the hydrolysis of FOGs, have shown ~30% loss of residual activity after the second reaction cycle, this poor reusability degree suggests that free lipases are not suitable for recycling even if they are recovered [133]. Consequently, the reusability of the immobilized enzyme is paramount to reduce operating costs and to improve

Table 5

Reported studies of enzymatic hydrolysis of waste FOGs.

Waste FOG	Lipase Source	Form	Optimum pH	Optimum Temperature [°C]	Oil:H_2O Molar ratio	Yield (%FFA)	Time [h]	Refs.
Used cooking oils (UCOs)	Candida rugosa	Immobilized	7.0	30	1:4.12	92.0[c]	30	[93]
UCOs	Candida rugosa	Immobilized	5.49	35.3	1:1[c]	99.7	2	[99]
UCOs	Thermomyces lanuginosus	Immobilized	Uncontrolled	30	1:4[c]	94.0	12	[95]
Waste corn oil	Tritirachium oryzae W5H	Free	5.0	33	1:5[c]	96.8[a]	12	[133]
UCOs	Candida Rugosa	Free	8.0	40	1:0.5[d]	85.0	3	[78]
UCOs	Candida Rugosa	Free	7.0	30	1:1[c]	62.9	24	[134]
UCOs	Araujia sericifera	Free	8.5	25	1:0.5[d]	60.0[f]	<1	[115]
UCOs	Candida Rugosa	Immobilized	7.0	50	1:10[d]	96.5[b]	1.5	[5]
UCOs	Candida Rugosa	Free	7.0	30	1:4 [c]	92.0[a]	30	[32]
Swine wastewater	Penicillium restrictum	Immobilized	6.0	45	1:99	100,1[e]	15	[135]
Palm oil mill effluent	Rhizopus oryzae	Immobilized	7.0	40	1:1[c]	89.51[a]	3	[91]
Soybean cooking oil	Candida Rugosa	Free	—	40	1:1.5[d]	—	3	[111]
Soybean soapstock	Lipozyme IM-20	Immobilized	8-10	42	1:3.75[d]	>99.0	20	[67]
Fish waste	Candida Rugosa AY30	Free	7.0	45	—	23.45[a]	6	[50]
Beef tallow	Rhizomucor miehei	Immobilized	7.0	40	1:9[c]	68.0[b]	72	[19]
Beef tallow	Candida rugosa	Free	—	30-35	1:1[d]	98.0[a]	24	[70]
UCOs	Candida rugosa	Free	—	30	1:1[c]	>93.0	12	[113]
Sunflower cooking oil	Pseudomonas otitidis	Free	7.5	35	1:29[c]	92.3	3	[116]
Olive pomace oil	Thermomyces lanuginosus	Free	—	35	1:0.25[d]	83.0[b]	24	[136]
soybean deodorization distillates	Candida rugosa	Immobilized	—	43	1:0.67[d]	94.0[a]	1.5	[35]
Acid Oil	Candida rugosa	Free	Uncontrolled	30	1:0.67[d]	90.0	24	[130]

[a] Hydrolysis degree [= (Av2-Av1)/(Sv – Av1) Av: Acid value Sv: saponification value].

[b] FFA % content.

[c] volume ratio.

[d] mass ratio.

[e] FFA concentration (μmol/mL).

[f] Yield to FFAs and MGs.

sustainability indicators [121]. In this case, the immobilization method (e.g. cross-linking, adsorption, encapsulation) influences the stability of the enzyme and dictates reusability [5,23]. Several studies on commercial immobilized lipases have established that their application in different processes is not economically feasible [100,117]. This occurs because inhibition, leaching and loss of immobilized catalyst within the reaction effluents prevents the required reuse in several cycles. Furthermore, hydrolysis reactions exhibit an extra issue because high water activity can reduce the lifetime of the catalyst [109]. Since water is a reactant, operating techniques and supports able to withstand high water content are mandatory to ensure good reusability. Additionally, high shear stress caused during mixing of reactive media also cause a loss of activity and enzymes leaching.

In order to enhance reusability, rinsing and washing with suitable solvents can help to remove unreacted substrates and products that may inhibit the enzyme, thus avoiding a decrease in its activity for further cycles. It has been observed that when no washing is carried out, a sudden decrease in the residual activity occurs [109]. In the selection of proper solvents, aspects such as hydrophobicity interactions, enzyme denaturing, and chemical interactions with the support have to be considered. Among the different assessed solvents (e.g. hexane, chloroform, acetone, water, ethanol, etc.), partially polar solvents (e.g. branched alcohols) are preferred because they can solubilize MGs, DGs, TAGs, and glycerol. The branched structure also avoids the formation of a water layer around the immobilized enzyme, facilitating the recovery with no activity loss [20]. It was demonstrated that Novozyme 435 can be re-used up to 50 cycles in hydrolysis reactions, by washing with tert-butanol, with no significant activity loss [137]. It might be pointed out that enzymatic hydrolysis generally presents a sudden decrease in the residual activity after a few cycles, and presumably this behavior is explained by the leaching of the lipase in the aqueous phase [95]. So, even when using a rinsing step with suitable solvents, it is expected that the activity of enzymes will be eventually reduced after certain number of cycles. Table 6 summarizes some reports about the reusability of enzymes in the hydrolysis of waste FOGs, illustrating a clear preference for the use of branched alcohols.

5. Prospects and future directions in waste FOGs hydrolysis

5.1. Developments in enzymatic processes

5.1.1. Pickering solutions

As previously mentioned, a major limiting factor in enzymatic processes is the lack of compatibility between reactants and the bioderived catalyst. Improvement of compatibility between immiscible or partially miscible reactants (e.g. TAGs hydrolysis) can be accomplished by using surfactants on the reactive media [138]. However, addition of such substances can cause separation issues in the downstream processing, and their removal from the final product can be cumbersome, resulting unattractive for industrial implementation [139]. Alternatively, there is an emerging interest in the development of enzymatic catalysts with the capability to enhance emulsification without the assistance of chemical additives (i.e. surfactants). This feature would be valuable to carry out reactions in immiscible or partially miscible media, and in systems where small droplet sizes are essential to ensure a large mass-transfer interface [28]. In the particular case of waste FOGs hydrolysis, this approach seems promising as the bioderived catalyst acts at the water-oil interface [20].

Table 6
Reported reusability studies of immobilized lipases in the hydrolysis of waste FOGs.

Lipase	Washing Solvent	Number of cycles	Residual activity (%)	Refs.
Lipozyme TL IM	Isopropyl alcohol	10	—	[21]
CRL/ Diaion HP-20	Tert-butanol	7	>99	[99]
Lipozyme TL IM	Tert-butanol	5	42	[95]
Novozyme 435	Tert-butanol	4	62	[27]
Pseudomonas gessardii on activated carbons.	Acetate buffer	21	100	[105]
CRL on hybrid sol-gel/ calcium alginate beds	No washing	4	55	[109]

The dispersion and stabilization of immiscible liquids has been done by mean of colloidal particles, which after enzymes doping, could also enhance hydrolysis by playing the double role of emulsifying agents and catalysts [139]. These types of systems are called Pickering emulsions, and because of their catalytic nature, they are referred as Pickering interfacial catalysts (PICs). Recent studies have stated that modified silicas grafted with active centers (e.g. enzymes) and alkyl chains have a better performance than homogenous catalyst commonly used in hydrolysis (e.g. H_2SO_2, TFA). Using this type of PICs in the hydrolysis of oils, it has been possible to obtain conversions of 94% with FFAs yields above 64% [138]. In the same way chitosan nanoparticles have exhibited an interesting biocompatibility with lipases immobilized by noncovalent cross-linking at neutral pH. This study proved that this catalytic system allows to obtain nearly complete conversion in 120 min in the hydrolysis of olive oil at 37 °C using 1:1 oil to water volume ratio. These results indicate the promising potential of this technology for the hydrolysis of FOGs [139].

5.1.2. Enzymes engineering, intracellular lipases, and novel immobilization trends

An enzyme with improved characteristics is desired to enable its use with non-conventional reactive media, such as waste FOGs [140]. For instance, a recent research on enzymes engineering developed multiple-site-mutagenesis of a CALB, achieving a lipase with enhanced stability and activity in non-conventional substrates. Mutant CALB probed that enhancing the flexibility of the binding region for the substrate can lead to an improved residual activity of 65%, even in high polar media [141]. Similarly, it has been demonstrated that the flexibility of the active site of a CALB is related to its activity in different substrates and solvents, so it was enhanced by modifying amino acids sequences in the helices near to the active site obtaining activities up to 5.4 fold the wild enzyme [142]. Part of the enzyme engineering is focused in the overexpression of the desired enzymes in microorganisms which exhibit a good culture performance (e.g. cell density, expression level). This has been reported in the expression of *Thermomyces lanuginosus* lipase (TLL) using Pichia pastoris yeast in the enzymatic hydrolysis of FOGs [131].

Even though the studies with extracellular lipases are more frequent, another way to improve the enzymatic processes is by using intracellular lipases by means of cell bound lipases [19]. This enables to reduce costs and to overcome the difficulties of enzymes extraction, purification and immobilization [143]. In this case, whole cells become the catalytic agents and they can also be immobilized to facilitate a continuous operation as in some fermentation processes. This has been studied in the hydrolysis of UCOs using TLL lipase, which allowed to obtain 93% FFAs content [131]. This approach has also been used in the hydrolysis of dairy products, employing *Penicillium* strains in mycelium-bond reaction systems [143]. Additionally, since the cell membrane is permeable and selective to specific molecules, immobilization of whole cells could prevent lipase inhibition by oxidized and polar impurities of waste FOGs [144].

These promising intracellular lipases can be employed in the hydrolysis of different waste FOGs, and future studies must be focused on optimizing culture conditions and suitable supports. When the objective is the immobilization of the whole cell, an alternative is the so-called passive cell immobilization with biomass support particles (BSPs). This technique is based upon the microorganism ability to colonize and grow in aggregates onto solid biomass particles [19]. Usually, the supports employed in the immobilization are polymeric foams made of polyurethane and polypropylene, and the immobilized lipases are obtained after cell cultivation and posterior liquid filtration with organic solvents. Finally, the activated foams are directly loaded into the reactive medium for fat hydrolysis. It has been demonstrated that lipases immobilized on BSPs have 2.1 to 4.3 times higher activity than free lipases on the hydrolysis of waste fats [19]. It has also been proved that *Penicillium restrictum* supported on babassu cake biomass has higher conversions than commercial immobilized lipases. These results could be due to the fact that commercial enzymes are purified lipases, whereas non-purified extracts and immobilized cells may have other hydrolase activities (e.g. proteases, amylases, esterases) which might enhance reaction performance. Additionally, biomass particles confer higher stability, as shown in wastewater treatments in which pools of hydrolases are more effective than pure enzymes extracts [135].

5.2. Combined processes involving enzymatic hydrolysis

5.2.1. Hydrolysis and esterification

Recent studies have evaluated the production of biodiesel in a two-step approach by mean of a hydro-esterification process. This involves the hydrolysis of TAGs followed by esterification of FFAs, and the corresponding process flow diagram is presented in Fig. 8 [99]. In this case there is no need for FFAs removal from the waste FOGs, but the methyl esters are readily produced under enzymatic processes [30].

Hydro-esterification of low-quality oils exhibit different advantages that have been recognized in the literature [3,95,99,113], such as:

- No need of water removal avoiding low yields of current trans-esterification process.
- Net water consumption is near to zero due to the fact that water consumed in the first hydrolysis step is recovered in the second esterification reactor.
- Milder conditions and thus a safer process can be developed
- Higher purity and easier purification
- High-purity glycerol recovered can be used in pharmaceutical and food purposes.
- Despite energy consumption is higher in the two-step process, it is compensated by higher yields and lower capital investment [145].

A recent study on biodiesel manufacture from waste FOGs, using the two-step approach and employing lipases produced intracellularly, concluded that it is more effective than the conventional alkaline process. This is because the cell membranes enable to reduce enzyme deactivation by polar inhibitors in the feedstock, and the system was more resilient to water content than the current transesterification processes [131]. Sundry studies have also indicated that lipases are the most suitable catalyst for hydro-esterification since lipases are substrate-specific, and are not affected by water or FFAs in the waste feedstock [95]. Although biodiesel has been the most studied product in the hydro-esterification process, other products have also been obtained from hydro-esterification of UCOs with maximum yields of 100% [32].

5.2.2. Hydrolysis and lipase production

Waste FOGs can be used as a low-cost substrate in biological cultures for the simultaneous production of lipases and/or fatty acids [98,116,146]. In this sense, oil cakes, agricultural residues and various industrial effluents have been used for the production of lipases because lipids and other related substances serve as inducers with high hydrolytic activity. In particular, enzymatic activities of 9.23 and 20.26 U/mL have been obtained during simultaneous hydrolysis and enzymatic production using olive oil mill effluent and palm oil effluent, respectively [147]. In this process, the presence of unsaturated fatty acids and minerals have exhibited improved hydrolytic activity and lipase production. Also, soapstocks from different sources have demonstrated being suitable substrates for lipase/FFAs production, since they play a role as extracellular lipase inducers in solid state fermentation (SSF) processes [66,148]. At the same time, it has been demonstrated that the consumption of FFAs by the Penicillium restrictum is negligible [135], indicating that FFAs produced by excreted extracellular lipases are not degraded by the same microorganism.

In a recent study, it was demonstrated that Yarrowia lipolytica yeast enables high lipase and hydrolysis yields using UCOs as substrate [149]. Likewise, the process was effectively verified using a culture of *Pseudomonas yamanorum LP2,* under non-sterile conditions. This demonstrated that impurities in UCOs do not generate lipase inhibition [150]. Several studies using soapstocks as sole carbon source for different microorganisms (e.g. *Aspergillus niger, Oospora lactis, Candida Rugosa*) have concluded that such waste FOGs are good inducers for the production of lipases with high activity (>7 U/mL), but with limited hydrolysis conversions [18]. Marine lipase strains have also been produced using UCOs and these exhibit a boosted hydrolytic activity and a broader pH and temperature stability [116]. All this confirms that the simultaneous

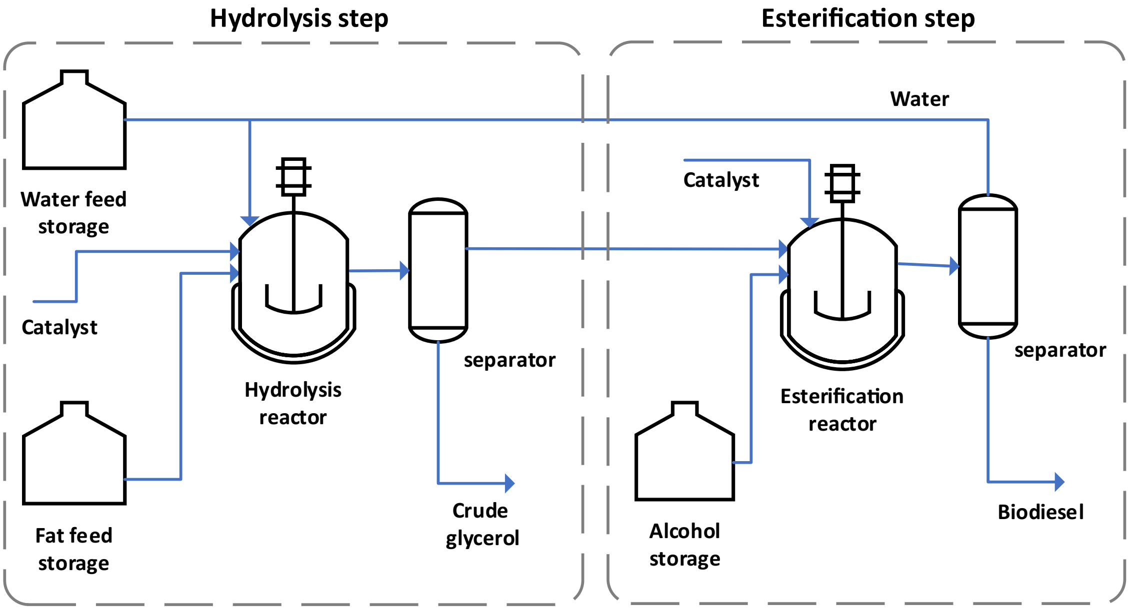

Fig. 8. Simplified process flow diagram for waste FOG hydro-esterification.

production of lipases and FFAs is a promising pathway to reduce operating costs during waste FOGs exploitation.

5.2.3. Hydrolysis and hydrogenation

Despite metals present poor catalytic activity in hydrolysis, and enzymes exhibit low activity in dehydrogenation, when combined they can enable the harnessing of waste FOGs for the production of FFAs and hydrogen. It has been proved that while lipases promote hydrolysis of waste FOGs, metal catalysts aid to convert the produced glycerol into other products (e.g. hydrocarbons, CO_2) releasing hydrogen [151]. This helps to minimize glycerol concentration in the educts of hydrolysis, thus improving the quality of the produced fatty acids [152]. A recent study explored the harnessing of beef tallow via hydrolysis and subsequent transformation of glycerol into hydrogen and CO_2, using NiO and PdO supported on $Al2O_3$ and SIO_2 [79]. An interesting feature of this process is that the produced hydrogen can simultaneously convert unsaturated fatty acids into saturated fatty acids [79]. However, in the case of tallow, the content of unsaturated chains is relatively low, hence, the major part of produced hydrogen could be exploited, making the process economically attractive.

5.3. Process intensification in waste FOGs hydrolysis

Process intensification appears as a promising way to overcome most mass transfer and kinetic limitations occurring during waste FOGs hydrolysis, to reduce energy consumption of the process, and to enable safer and cleaner operating conditions [153]. A variety of methods, equipment, and processes have been explored in the enzymatic hydrolysis of FOGs, including assisted activation techniques (e.g. microwaves, ultrasound) and novel reactor configurations (e.g. microreactor, multifunctional reactors, reactive distillation), among others [119]. In this case, the choice of equipment and operating configuration should be oriented to avoid limitations of the hydrolysis process (e.g. mass transfer limitations, solubility, impurities in the feedstock, chemical equilibrium). Then, intensified technologies can help improving product quality, decreasing equipment size, reducing energy and materials intensity, electrifying processes, simplifying production schemes, and mitigating emissions; all resulting in cheaper, safer, and more sustainable processes [153].

5.3.1. Cavitation-assisted methods

This technique involves processes in which minute gaseous void cavities are generated and suddenly collapsed within the liquid phase. A particularity of this phenomenon is that it may occur simultaneously at several locations in the reactive medium. Beside, cavitation can release a vast amount of energy in high densities, which highly increases temperatures in small volumes and boosts mass transfer rates and reaction kinetics [119]. It has been verified that generation of localized hotspots in the reactive medium boosts hydrolysis reactions, even without the use of catalysts [82]. This phenomenon is unchained when the local pressure of the molecules is less than vapor pressure, so cavities are generated by sudden pressures changes [28].

Currently, four cavitation methods are commonly employed: acoustic, hydrodynamic, optical, and particle. However, in the industrial practice, hydrodynamic and acoustic are the most employed since particle and optical cavitation are not suitable for reactions [28]. In hydrodynamic cavitation, cavities are generated by a variation in the system geometry (e.g., using a venturi), causing a sudden change in the velocity [154]. On the other hand, ultrasound and microwaves induce acoustic cavitation by a local pressure change [27,155]. A remarkable advantage of cavitation is that enables shorts times for reaction and emulsification, which simplifies downstream separation of products under milder conditions [119]. Emulsification by cavitation also exerts positive effects in FOGs hydrolysis considering the surfactant nature of intermediate and final products. Since FFAs, MGs, and DGs are surface-active molecules that tend to accumulate in the water-oil interfaces, cavitation can enhance emulsification by reduction of droplet sizes, improving mass transfer and hydrolysis rate [20]. Beside, as the produced glycerol increases droplet sizes affecting mass transfer, cavitation can help overcoming this issue by inducing droplets rupture.

Cavitation may be induced in the system by an ultrasonic bath or a probe. While baths are effective for small volumes, probes have demonstrated better performance in larger reactors [156]. Still, the main challenge of acoustic cavitation systems is their scalability for industrial process since energy is highly dissipated when working with large volumes [119]. In this regard, hydrodynamic cavitation using controlled flow cavitation and shock power reactors could be employed [157]. Despite a recent study concluded that TAGs hydrolysis of oils is slightly influenced by hydrodynamic cavitation [158], other researchers affirm that there is pending research on different geometries and cavitation devices for these reactions [154].

Ultrasound irradiated to a fluid at frequencies above 20 kHz using a transducer (e.g. horn, probe, plate) can deliver high energies [82]. In the case of hydrolysis reactions this promotes a greater homogeneity, increasing interface area between oil and water, thus resulting in high conversions of TAGs even at low water loadings in relative short time [20,24]. Although ultrasound increases hydrolysis rates, its use must be further studied because disturbances could lead to conformational, structural changes, or desorption of enzymes from the support. However it has been found that enzymatic hydrolysis at high biocatalyst concentrations is favored by ultrasound due to the dispersion of formed enzymatic clusters [20]. In the same way, it has been observed that the use of mechanical stirring alternated with ultrasound provides a five-fold increase of reaction rates and higher conversion when compared with simultaneous ultrasound and mechanical stirring [27]. For this reason, some studies have evaluated the use mycelium-bond lipases under ultrasound influence, reaching up to 96% hydrolysis yields [143]. A comprehensive summary of most recent studies on the enzymatic hydrolysis of waste FOGs under ultrasound action is presented in Table 7. For comparison purposes, a specific work reporting ultrasound-assisted hydrolysis of waste FOGs with traditional inorganic homogeneous catalysts also permitted to verify high yields at reduced temperatures [83].

As observed, ultrasound-assisted enzymatic hydrolysis of residual TAGs is a promising way to enhance reaction performance under milder conditions. Additionally, the use of ultrasound technology enables to work in solvent-free systems since ultrasound confers good mixing without using emulsifiers or solvents. This is a major benefit when considering the industrial implementation of hydrolysis to exploit waste FOGs [24,27]. However, for enzymatic processes, continuous exposure of ultrasonic could lead to severe damage to the tertiary structure of the enzyme, causing deactivation and damage due to the ultrasonic transducer [27,159]. In this case, the use of immobilized enzymes would be more effective. Interestingly, it has been reported that ultrasound can also be employed in enzyme immobilization, substrate pre-treatment, and/or carrier functionalization [160].

In the case of microwave reactors, they are perhaps the less studied acoustic cavitation technique for TAGs reactions [28]. One of the first attempts to develop microwave-assisted enzymatic hydrolysis for DGs production resulted in yields lower than 30% [161]. The poor results can be explained because microwaves tend to heat the reactive media instead of favoring cavitation and mass-transfer improvements [119]. Nevertheless using microwave irradiation in the self-catalytic hydrolysis of soybean oil at 195 °C and ambient pressure, conversions up to 96.6% were achieved, avoiding the limitations of the current self-catalytic Colgate-Emery process [155]. However, the combination of microwaves and ultrasound could help overcoming individual limitations [162]. Some studies have disclosed that this combination avoids mass transfer limitation of microwaves and heat transfer limitation of ultrasound [163].

Table 7
Recent studies on the ultrasound-assisted hydrolysis of waste FOGs.

Catalyst	Frequency [kHz]	Power [W]	Temperature [°C]	oil:water molar ratio	Yield (% FFA)	Catalyst concentration (% wt.)[a]	Time [h]	Refs.
Lipozyme TL IM	40.0	132	40.0	1:20	60.68 [g]	10.0	2	[20]
Novozyme 435	22.0	100	50.0	3:1	75.19	1.25	2	[27]
Aspergillus Nigger	40.0	66	45.0	1:3[f]	62.67 [e]	15.0 [b]	12	[159]
Aspergillus Nigger	40	66	45	1:3[f]	753.07[e]	15.0 [b]	12	[24]
Pseudomonas gessardii on activated carbons	20.0	–	35.0	1:100>	∼100 [c]	1.66	4	[105]
KOH/Sulfuric acid	42.0	160	60.0	1:2.4 [h]	97.3	0.3 [d]	<1	[83]

[a] by weigh of substrates.
[b] % v/v.
[c] % hydrolysis [= (Av2-Av1)/(Sv – Av1) Av: Acid value Sv: saponification value].
[d] Catalyst to oil ratio.
[e] FFA concentration (μmol/mL).
[f] Volume ratio.
[g] FFA %p/p in the reaction sample.
[h] Oil:ethanol mass ratio.

5.3.2. *Intensified reactor configurations*

Catalyzed-hydrolysis performance can be enhanced by implementing novel configurations or continuous reaction schemes (e.g. Micro-reactors, membrane reactors, oscillatory baffled reactor, etc.). These types of reactors have been successfully implemented in a wide range of applications in suspensions or catalytic reactions [28]. Nonetheless, despite reaction performance might be enhanced, adversely effects might appear in the separation steps and products recovery, since some of these techniques tend promote emulsions to obtain high mass-transfer rates. Then, such secondary effects should be considered if a certain reactor configuration would be deployed for the hydrolysis of FOGs. In this case, reactors with high contact area per unit of reactive volume operating under low turbulence could be considered. Also, simultaneous reaction-separation in centrifugal spinning disc reactors would facilitate separation of immiscible phases. Although the evidence suggests that such intensified reactors could benefit the industrial-scale implementation of enzymatic process, there are few reports in the open literature dealing with the enzymatic hydrolysis of waste FOGs.

Micro-reactors

Micro-processes have received attention during last years because they facilitate continuous operation under highly efficient heat and mass transfer. Microstructured reactors or microreactors consist of micro-channels of small size (10–500 μm), internally grafted with diverse materials such as polymers, glass, metals, and ceramics [164]. This type of configurations provides large surface area to volume ratio, and facilitates mixing on a microscale. The corresponding mixing times in microreactors are considerably shorter than in conventional reactors, which aid to overcome mass-transfer limitations [119]. They also generate less waste streams due to the better use of reactants, also enabling simultaneous reactions and separations [165].

A recent work reported the hydrolysis of TAGs by using immobilized-lipase on nanoporous materials, and it was found that the activity in microreactors is higher than in batch reactors [166]. A similar work studied the hydrolysis of a vegetable oil in microfluidic systems, and despite conversions up to 50% were observed, results indicate that a fast production can be carried out with less energy consumption and easier products separation [167]. In this case, it was suggested that reaction performance could be enhanced by adopting a massive parallelization approach.

Membrane reactors

Membranes are semi-permeable barriers that differentiate species of a solution by size exclusion, restricting the passage of some components. This characteristic depend tightly on the material, geometry, and structure of the membrane [121]. Membrane separation technology is attractive in many fields and it can be coupled with reaction steps, reducing the cost in downstream purification stages [86]. Some authors have demonstrated that this technology is suitable for hydrolysis of fats, because the selective removal of glycerol from the reaction medium enables yields of FFAs up to 94.5% [86]. Likewise, sundry studies confirm that lipases absorbed onto hydrophobic membranes showed suitable performance in hydrolysis reactions [168], with faster reaction rates compared with other reaction schemes. These immobilized enzymes also exhibited a high activity and stability in continuous operation for several days [169]. Despite the promising performance, the main limitations of membrane reactors for the enzymatic hydrolysis are related to diffusional limitations and enzyme-inhibition phenomena owing to the fouling and clogging of the membranes with impurities [170]. Additionally, while the technology would be very effective in the hydrolysis of waste FOGs, there is need for a better understanding of the effect of the many impurities on the membrane fouling and enzymes inhibition [171].

Oscillatory baffled reactor

The oscillatory baffled reactors (OBRs) are continuous flow reactors consisting of a tube with internal spaced orifice plate baffles that operates in a pulsed flow rate. This operating mode favors recirculation flow near the baffles, thus enhancing mixing, and consequently mass and heat transfer [172]. OBRs have been successfully employed in several biphasic reactions such as esterification, transesterifications, and epoxidation of virgin and waste oils [173,174]. In the particular case of waste FOGs hydrolysis, OBR can highly increase the interfacial area between the reactants enhancing reaction yields [28]. Nevertheless, further studies are needed because high shear rates in OBR may play a limiting role in enzymatic reactions due to inhibition by shear stress or enzyme leaching.

Other intensified reactor configurations

Different intensification approaches have been evaluated to enhance FOGs hydrolysis, for instance by using high voltage electrical fields [175]. This technique facilitates mass transfer rates owing to enhanced convection within and around the drop, due to an increase in the specific area and the turbulence [176]. A recent work evaluated the hydrolysis of a crude vegetable oil using Immobilized *Candida Rugosa*, and it was concluded that the process was indeed enhanced by the influence of an external electric field [177]. Additionally, novel equipment such as spray columns has been studied in enzymatic hydrolysis of oils exhibiting suitable reaction performance [178]. However additional studies must be conducted to assess the impact of impurities in waste FOGs on the system performance.

6. Conclusions and future directions

Different aspects of the hydrolysis of waste FOGs were presented mainly focusing in enzymatic catalyzed processes. An analysis of second-generation feedstocks, chemical routes, catalysts, process limitations, operating conditions for enzymatic processes, immobilization techniques, combined reactions, and current intensification approaches were covered. From the revised literature it was evidenced that there is a suitable global supply of different waste FOGs that can be used as alternative feedstocks for the oleochemical industry. As they are mainly composed of TAGs and FFAs, waste lipids such as used cooking oils, trap greases, soapstocks, vegetable oil extraction and refining residues, and food wastes among many others can provide a great fraction of lipidic feedstocks used in current biofuels and oleochemical market. Beside reducing waste disposal problems, the exploitation of such waste streams can improve sustainability indicators of the oleochemical industry by embracing circular economy production schemes. In particular, transformation into high-value added products are of major interest in order to ensure long-term sustainability of FOGs reclaiming and valorization schemes.

Despite the great potential, major challenges of exploiting low-quality feedstocks include their characteristic high content of acidity and moisture. In this regard, complete hydrolysis of TAGs and partial glycerides into FFAs is a suitable route for the complete recovery and exploitation of waste lipids. While the valorization of waste FOGs can be implemented using existing hydrolysis technologies, these are highly energy and resources intensive, operate under hazardous conditions, and can have major impacts in the quality of the products. Alternatively, it was demonstrated that enzymatic hydrolysis with lipases can overcome major limitations of the traditional processes, enabling to operate under milder conditions even with the highly heterogeneous waste FOGs. Different lipases were identified as suitable for the industrial implementation of such processes, but considering the high heterogeneity of the waste feedstocks, non-specific enzymes (e.g. *Candida Antarctica Lipase B*) and highly resistant ones (e.g. engineered and marine enzymes) were identified as the most promising catalysts. Beside, enzymes immobilization into mesoporous materials is envisioned as a suitable technique to improve enzyme activity and reusability, and to overcome major drawbacks of traditional enzymatic hydrolysis.

In addition to the above, most recent advances in enzymatic hydrolysis of FOGs were presented, including new catalysts development, immobilization methods, the use of Pickering solutions, intracellular lipases, and combined chemical reactions. Based upon these studies, it was observed that simultaneous-sequential enzymatic hydrolysis and esterification using a bifunctional enzyme is a promising route for the valorization of waste FOGs. Also, after recognizing major limitations of the enzymatic hydrolysis processes, most promising intensification techniques were identified. In this case, ultrasound and microwave devices and microreactors can be successfully used to enhance reactants compatibility, transport phenomena and reaction kinetics in the hydrolysis of waste FOGs. Finally, despite the variety of investigations that have been conducted in the revised field, further studies are still required to assess energy intensity, catalyst reusability and stability, process resilience to enable highly heterogeneous feedstocks, and intensification schemes for the enzymatic hydrolysis at the pilot and industrial scale.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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