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Hierarchical zeolites as catalysts for biodiesel production from *Nannochloropsis* microalga oil

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ABSTRACT

Oleaginous microalgae are potential sources for biodiesel production as they accumulate high levels of lipids (>20%) and they do not compete with food production. The lipid extraction from *Nannochloropsis gaditana* was studied using three procedures (sonication, microwaves and magnetic stirring under reflux) with different extraction solvents (methanol, chloroform/methanol and hexane). The extraction with methanol and magnetic stirring under reflux gave the highest cell disruption and therefore, the highest lipid extraction. The extracted lipids were characterized to determine the fatty acid profile.

On the other hand, microalga lipids were tested as a feedstock in the biodiesel production using hierarchical zeolites as heterogeneous catalysts, to overcome the disadvantages of homogeneous catalysts currently used in the industrial plants. Hierarchical Beta zeolite showed a significant activity in the microalga oil reaction with methanol, because it presented a secondary porosity in the range micro-mesopores maintaining the acid properties of the Beta zeolite. In this sense, the diffusion restrictions to the mass transfer of large lipids into zeolite framework were reduced, improving the accessibility of microalga oil to the h-Beta acid sites.

1. Introduction

The contribution to the global warming of the combustion of fossil fuels is one of the main environmental problems. Furthermore, the well known oilfield exhaustion is remarkable. Biodiesel production from vegetable oils has been developed as an alternative for fossil fuels. However, biodiesel presents some disadvantages. One of its drawbacks is the high manufacturing cost, which is due to the high cost of the vegetable oil. In addition, the biodiesel industry competes with the food industry for oil crops. In fact, it needs large percentages of the current available arable land to obtain biofuel using crops such as rapeseed or sunflower [1]. Therefore, it is necessary to explore new raw materials that reduce the biodiesel price without competing with food production. Moreover, biodiesel from oilseed crops, waste cooking oil and animal fat does not satisfy the existing demand for transport fuel and, therefore, other alternatives should be studied [2,3]. Lipids obtained from different microorganisms (microalgae, bacteria, fungi and yeast) are being studied in biodiesel production since these oleaginous microorganisms can assimilate carbohydrates and accumulate high levels of intracellular lipids (>20%) [4–11]. Besides, microorganisms can be grown in nonarable land so that they do not compete with food production. It has been reported that these microorganisms could accumulate different lipid contents, depending on their culture conditions (temperature, pH, light intensity, etc.) and genetic factors [4].

Specifically, the feasibility of microalgae to produce biodiesel is being taken into account, as an alternative source for fossil fuels [12], since they are rich in lipids and can increase twofold or more times their biomass within one day [11]. As well, the biodiesel production from these microorganisms shows many advantages over the production from oilseed crops including the higher oil productivity per area of microalgae culture, the potential modulation of the biochemical composition (oil content) by modifying the growth step and the higher rates of CO_2 fixation by the algae [13].

In particular, species like *Chlorella vulgaris*, *Chlorella emersonii*, *Nannochloris* sp., *Nannochloropsis* sp., *Neochloris oleoabundans*, *Phaeodactylum tricornutum* and *Tetraselmis sueica* have been reported in the literature, because they can accumulate important lipid amounts [2].

Nannochloropsis species (Eustigmatophyceae) show total lipid contents from 10 to 60 wt% in dry matter [2,14–16]. Oil productivity, that is the mass of oil produced per unit volume of the microalgal broth per day, depends on the algal growth rate and the oil content of the biomass. Microalgae with high oil productivities are desired for producing biodiesel [2], which includes cultivation, harvest, lipid extraction, and lipid transesterification.

The transesterification of triglycerides (the main component of vegetable oil) produces monoalkyl esters of long-chain fatty

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acids with short-chain alcohols [4]. The triacylglycerols (triglycerides) consist of three long chain fatty acids esterified to a glycerol backbone. When the triglycerides react with an alcohol (the most commonly used is methanol due to its low cost), the three fatty acid chains combine with the alcohol to yield fatty acid alkyl esters, which constitute the biodiesel (e.g., fatty acid methyl esters or FAMEs). This reaction is catalyzed by acids, alkalis and lipase enzymes [2]. The transesterification using enzymes has been reported to be very expensive (the enzyme costs are very high), shows deactivation problems and requires a much longer reaction time [17]. On the other hand, acid and basic transesterification are widely used for biodiesel production. It is well known that basic catalyzed transesterification is faster than the acid catalyzed reaction (about 4000 times). However, acid catalysts can simultaneously promote esterification of free fatty acids (FFAs) and transesterification of triglycerides [17–19]. Traditionally, homogeneous catalysts have been used for both acid and basic catalyzed reaction. Sulfuric acid is the main acid catalyst used for biodiesel production [20] whereas NaOH, KOH, Na_2CO_3 mixed with alcohol, are commonly used for homogeneous basic catalysis [17,21]. However, one of the major disadvantages of homogeneous catalysts is that they cannot be reused or regenerated, because they are consumed in the reaction and separation of catalysts from products is difficult and requires more equipment, which could result in higher production costs. Besides, the process is not environmentally friendly because a large amount of wastewater is produced in the separation step. Based on the above premises, the use of solid catalysts seems to be an appropriate solution to overcome problems associated with homogeneous catalysts. Nevertheless, one of the major problems associated with heterogeneous catalysts is the formation of three phases with alcohol and oil, which leads to diffusion limitations thus lowering the rate of the reaction [22,23].

Basic solids like CaO and MgO supported on alumina [24], and hydrotalcites [25] have been used for biodiesel production from vegetable oils. To avoid diffusion limitations, catalysts with higher surface area (porous silica-metal oxide composites) were tested in the transerification of vegetable and animal oils providing high conversion to (C10–C30) alkyl methyl esters and glycerin [26].

On the other hand, zeolites, ion-exchange resins, mixed metal oxides or mesostructured solids have shown promising results in the acid esterification and transesterification of vegetable oil with high content of free fatty acids (FFAs) to obtain FAMEs [27–30]. Recently, the transesterification of triglycerides contained in waste oilseed fruits with methanol has been studied using zeolites as strong acid catalysts (USY, BEA, FAU-X), together with weak acid catalysts (siliceous MCM-41 and ITQ-6) and base catalysts such as K-MCM-41 and K-ITQ-6 [29].

Zeolites are microporous crystalline metallosilicates featured by exhibiting molecular sieve and shape selective properties, which have found widespread applications in catalytic, adsorption, and ion exchange processes. Zeolites have usually been synthesized with crystal sizes in the micrometer range and, therefore, with negligible external surface area. These properties impose severe limitations for their use in the conversion of bulky compounds.

A huge interest has emerged recently for the synthesis of new zeolitic materials with enhanced accessibility. In this sense, nanocrystalline hierarchical zeolites contain a bimodal porosity (micro- and mesopores) and high external surface area where active sites can catalyze reactions involving large molecules like tryglicerides. The synthesis of hierarchical nanozeolites is based on the incorporation of organosilanes in the synthesis gel to prevent zeolite crystal growth and thereby to stabilize zeolitic particles with ultrasmall sizes [31].

In this context, it could be interesting to use ZSM-5 and Beta hierarchical zeolites [31] as acid catalysts in the transesterification reactions of *Nannochloropsis gaditana* oil to produce biodiesel. This work also includes the lipid extraction and the biodiesel production from microalga oil. The influence of the extraction method performed with different solvents was studied in order to obtain the highest lipid amount. After the extraction step, ZSM-5 and Beta zeolites were tested at different temperatures for biodiesel production and compared to sulfuric acid as conventional homogeneous catalyst.

Table 1
Different methods for microalga lipid extraction (time = 60 min).

Method	Temperature (°C)	Solvent
Sonication	45	M C:M H
Microwaves	45	M C:M H
Magnetic stirring under reflux	Boiling temp.	M C:M H

2. Materials and methods

2.1. Oil extraction

Lipids from the dry cell biomass samples of *N. gaditana* were extracted using three different solvent systems: chloroform:methanol (C:M; 2:1 v/v), methanol (M) and n-hexane (H) and different procedures. Sonication, microwaves and magnetic stirring under reflux were chosen, as they are the main methods described in the literature for lipid extraction [1,32,33]. The extraction conditions are summarized in Table 1. The extraction solvents, chloroform (>99%), methanol (>99%) and n-hexane (>96%), were purchased from Scharlab (Madrid, Spain).

Free fatty acids, triglycerides, diglycerides, monoglycerides, FAMEs, carotenoids, sterol esters, sterols and tocoferols, retinoids and polar lipids from the extracted oil were identified and quantified by Thin Layer Chromatography (TLC) analysis. Chromatographic separation was developed in 20 cm × 20 cm silica-coated aluminum plates (Alugram Sil G/UV Macherey-Nagel GmbH, Düren, Germany) using a solvent mixture of 88 vol% n-hexane, 11 vol% diethyl ether and 1 vol% glacial acetic acid. Visualization was carried out by staining with iodine. Digital image analyses of staining plates were performed with Un-Scan-It Gel 6.1 software (Silk Scientific Inc. Orem, UT, USA) and the lipid compositions were quantified by the corresponding calibration curves [1].

Fatty acid profiles of microalga oil were performed by gas chromatography in a CP-3800 gas chromatograph (Varian Inc.) fitted with FID detector and ZB-WAX capillary column (30 m length, 0.32 mm internal diameter; 0.25 μm film thickness Phenomenex, USA). Prior to GC analysis, the oil samples were transformed into their corresponding methyl esters using the boron trifluoride method described in the EN ISO 5509. Finally, 1 μl of the sample containing FAMEs was injected into the capillary column where the separation was achieved using a temperature ramp (1 °C min^{-1}) from 150 °C to 240 °C at a flow rate of 1 ml min^{-1} (injector temperature: 180 °C, detector temperature: 280 °C, injection mode: splitless). Identification of chromatographic peaks was performed by comparison with a FAME standard mixture and quantification by means of external standards and their corresponding calibration curve.

2.2. Catalyst preparation and characterization

Hierarchical ZSM-5(h-ZSM-5) and Beta (h-Beta) zeolites were prepared from organofunctionalized seeds, following the earlier procedures reported by Aguado et al. [34] and Serrano et al. [31], respectively. This method is based on perturbing the growth of the zeolite crystals by functionalization of the zeolitic seeds with organosilanes in order to hinder and prevent their further aggregation and agglomeration. The precursor solution of MFI or BEA units were precrystallized and the gels obtained were mixed with the organosilane (Phenylaminopropyltrimethoxysilane (PHAPTMS, 97 wt%, Aldrich) (C_6H_5)NH(CH_2)3Si(OCH_3)$_3$), the resulting mixture being kept in a reflux system under stirring (100 rpm) at 90 °C for 6 h. The organosilane was added in a proportion of 12 and 8 mol% in regards to the silica content in the synthesis gel to obtain h-ZSM-5 and h-Beta, respectively. Thereafter, the crystallization of the functionalized seeds was carried out in a stainless steel reactor under autogenous pressure. The solid products obtained were separated by centrifugation, washed several times with distilled water, dried overnight at 110 °C and calcined in air at 550 °C for 5 h. In order to clarify the effect of the improvement on textural properties of the hierarchical zeolites, the conventional ZSM-5 and Beta zeolites were also synthesized using the procedures described in Refs. [35,36], that is, the same procedure described for hierarchical materials omitting the silanization step.

Furthermore, the influence of the different textural properties of these materials was studied in biodiesel production to achieve high FAME yields. Analytical grade 95–97% sulfuric acid (provided by Scharlab) was used to compare the synthesized solids with a homogeneous catalyst.

All the solid materials were characterized by the following techniques. XRD measurements were taken in a Philips X'PERT MPD diffractometer using Cu Kα radiation with step size and counting time of 0.02° and 10 s, respectively. Argon adsorption–desorption isotherms, were measured at 87 K with an Autosorb instrument (QUANTACRHOME) [37]. The Si/Al atomic ratio of these samples was determined by ICP-AES measurements with a Varian VISTA-AX-CCD equipment. In order to determine the strength and number of acid sites, ammonia temperature programmed desorption (TPD) experiments were made in a Micromeritics 2910 (TPD/TPR) apparatus.

2.3. Biodiesel production

The catalytic experiments were carried out in a 0.1 l stirred batch autoclave, equipped with a temperature controller and a pressure gauge under stirring (1000 rpm) and autogenous pressure. Algae oil, methanol as solvent (100 l^{-1} methanol to lipids molar ratio) and catalyst (2 wt% of oil + methanol feed) were introduced in the glass reactor. The reactor was then immersed in a thermostatic bath at the reaction temperature for 4 h. Different temperatures (T = 85, 100, 115 °C) were tested for biodiesel production. Then, the FAMEs layer was collected and the crude glycerol was washed five times with n-hexane:diethyl ether (80:20) and the same volume of water. The upper organic layers were put together with the first FAMEs layer and the solvent was removed in a rotary evaporator leaving the residue containing the FAMEs, which was used to the qualitative characterization by TLC previously described.

3. Results and discussion

3.1. Oil extraction

Fig. 1 shows the lipid content extracted from *N. gaditana* with different solvents (methanol; chloroform and methanol; and hexane) and extraction procedures (sonication, microwaves and magnetic stirring under reflux). It can be seen how the highest lipid recovery was reached using a magnetic stirring under reflux regardless the chosen solvent. This can be justified as this method causes a cell disruption stronger than sonication and microwaves. However, Lee et al. [32] had reported that the microwave oven method showed the highest oil extraction efficiency for *Botryococcus* sp., *C. vulgaris*, and *Scenedesmus* sp. microalgae.

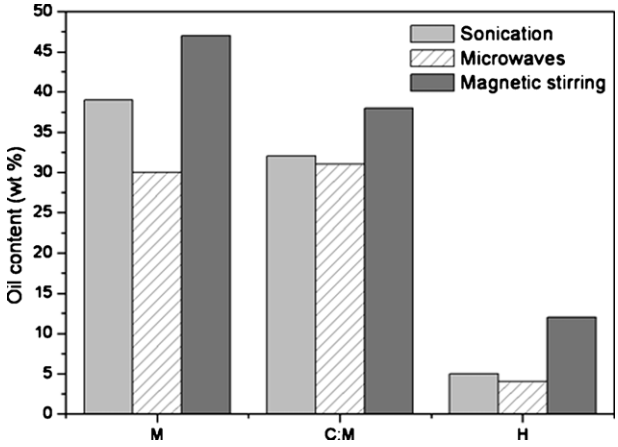

Fig. 1. Comparison of different solvents and procedures for microalga lipid extraction.

The highest extracted oil amounts were achieved using methanol as solvent (39, 30 and 47 wt% for sonication, microwaves and magnetic stirring, respectively). The amount of oil decreased when chloroform and methanol were used as solvents (32, 31 and 38 wt% for sonication, microwaves and magnetic stirring, respectively). On the other hand, the extracted oil seemed to be the lowest for the extractions made using hexane (<15 wt% for all the methods). The content of polar lipids (phospholipids, sphingolipids, and saccharolipids) was determined by TLC. Using a magnetic stirring under reflux, the methanol extracted the highest polar lipids amount (56 wt%), followed by the mixture chloroform:methanol (~31 wt%) and hexane (~9 wt%), This could be explained by the different polarities of the solvents following the order: methanol > chloroform:methanol > hexane. Therefore, hexane may not be able to extract polar lipids from *N. gaditana* microalga and methanol seems to be the most suitable solvent. Furthermore, the use of methanol and n-hexane hasgabeen suggested as an interesting alternative to avoid the use of chlorinated solvents (as chloroform), because of the adverse effect of these solvents on the environment [1].

The method selected to provide high lipid extraction was the magnetic stirring of microalga biomass with methanol under reflux. The extracted lipids were characterized by gas chromatography to determine the fatty acid profile. These results are summarized in Table 2. As can be seen, *N. gaditana* oil contains monounsaturated fatty acids and saturated fatty acids in relatively high concentrations. Particularly, large amounts (around 30 wt%) of palmitic (16:0) and palmitoleic (16:1) acids were found. In addition, the microalga oil also contained a significant amount (17 wt%) of a polyunsaturated fatty acid like eicosapentaenoic acid (20:5). These results are in agreement with those reported in literature [14].

3.2. Catalyst characterization

As illustrated in Fig. 2, synthesized zeolites were highly crystalline although after silanization hierarchical samples, denoted as h-ZSM-5 and h-Beta, presented less intense XRD peaks, suggesting the presence of smaller crystalline domains.

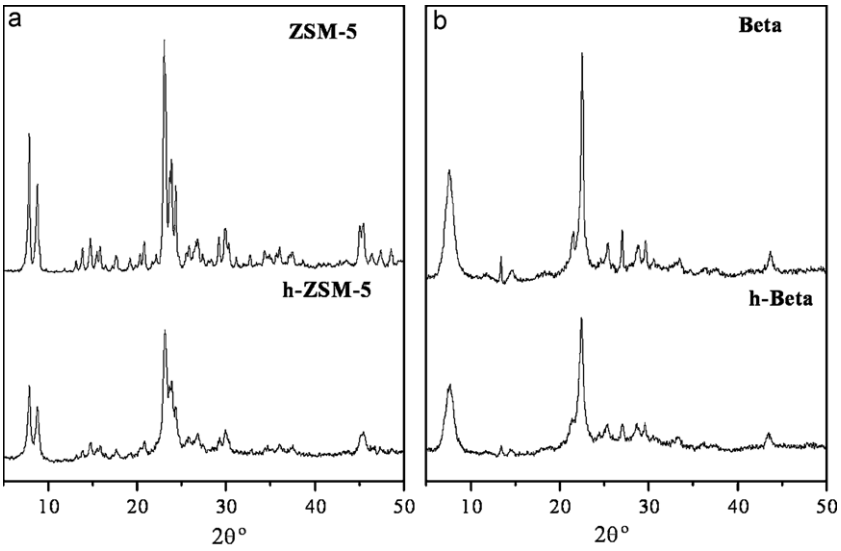

Fig. 2. XRD difractograms of (a) ZSM-5 calcined zeolites. (b) Beta calcined zeolites.

Table 2
Fatty acid composition of the extracted lipids from *Nannochloropsis gaditana*.

Fatty acid		Concentration (wt%)
Lauric acid	12:0	n.d.
Myristic acid	14:0	5.3
Myristoleic acid	14:1	1.1
Pentadecanoic acid	15:0	n.d.
Palmític acid	16:0	33.2
Palmitoleic acid	16:1	28.0
Stearic acid	18:0	2.1
Oleic acid	18:1	6.0
Linoleic acid	18:2	n.d.
Linolenic acid	18:3	n.d.
Arachidic acid	20:0	n.d.
Gadoleic acid	20:1	n.d.
Eicosapentaenoic acid	20:5	16.9
Erucic acid	22:1	1.4
Lignoceric acid	24:0	n.d.
Nervonic acid	24:1	n.d.
Other		6.2

The physicochemical properties of conventional and hierarchical zeolites synthesized in this work are summarized in Table 3. Conventional zeolites had significant Al amounts, with Si/Al ratios very close to the synthesis gel (Si/Al = 30). Although hierarchical zeolites were synthesized from silylated, seeds, the amounts of silicon added with the organosilane (PHAPTMS) were very low (see Section 2.2) and, therefore the Si/Al ratios of hierarchical zeolites were very similar to the conventional ones.

Fig. 3(a and c) and Table 3 evidence an increase in both total surface area and the pore volume of the hierarchical zeolites. This enhancement of the textural properties was caused by the incorporation of the organosilane anchored on the zeolite seeds. Thus, while the external surface area increased with silanization agent, the secondary porosity was clearly enhanced by the seed silanization treatment. It is noticeable the larger increase in the external surface area experimented by ZSM-5 upon silanization. In fact, h-ZSM-5 sample had almost 60% of the total BET area as external surface area. Besides, both zeolites presented the typical pore size distribution centered at 5.22 and 5.76 Å for ZSM-5 and Beta respectively (Fig. 3b and d). However, the silanization process is responsible of the development of a secondary porosity in the range of micro-mesopores. In this sense differences were found between both hierarchical zeolites with h-ZSM-5 secondary pores between (15–154 Å) and h-Beta between (16–54 Å).

The acidic properties of these zeolitic materials were measured by ammonia TPD experiments and the results are shown in Table 3. In general, the acid strength and acid sites population decreased in silylated materials being more evident in h-ZSM-5 sample with only (0.33 mmol/g) while h-Beta retained almost the original acidity and acid strength of Beta zeolite with much higher surface area (mainly external surface area) and pore volume.

3.3. Biodiesel production

The high concentration of free fatty acids and polar lipids in the *N. gaditana* oil determines that an acid-catalyzed process is more suitable for producing biodiesel than an alkali one to avoid yield losses. The microalga oil extracted with methanol under reflux and magnetic stirring was used in the biodiesel production with both homogeneous and heterogeneous acid catalysts at different reaction temperatures (T = 85, 100, 115 °C). Sulfuric acid was tested as homogeneous catalyst whereas conventional and hierarchical

Table 3
Physicochemical properties of the zeolite catalysts.

Sample	S_{BET} (m^2/g)	S_{EXT}[a] (m^2/g)	Vpore (cm^3/g)	$V_{\mu p}$[a] (cm^3/g)	Si/Al[b]	T_{max}[c] (°C)	Acidity[c] (mmol/g)
ZSM-5	431	78	0.6234	0.2031	31	357	0.4259
h-ZSM-5	696	456	0.8725	0.1334	32	329	0.3303
Beta	599	87	0.4316	0.3039	27	321	0.4434
h-Beta	725	287	0.6451	0.2600	28	316	0.4236

[a] $S_{EXT} = S_{BET} - S_{\mu P}$, $S_{\mu P}$ and $V_{\mu P}$ obtained by NLDFT over Ar isotherm.
[b] ICP-AES.
[c] From NH_3-TPD measurements.

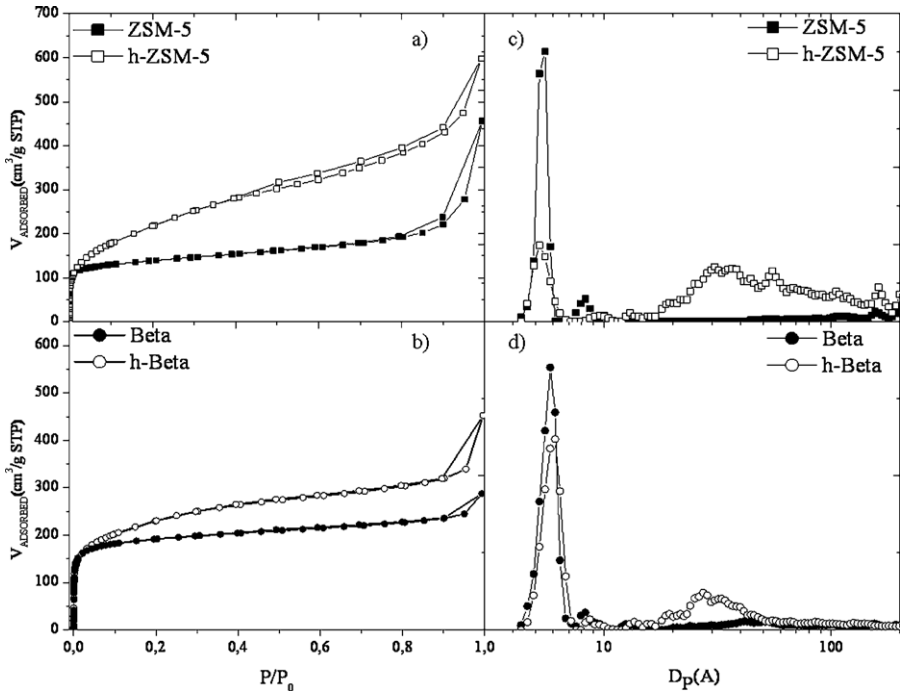

Fig. 3. Ar adsorption–desorption isotherms of (a) ZSM-5 samples, (b) Beta samples. Pore size distribution obtained by NL-DFT of (c) ZSM-5 samples, (d) Beta samples.

zeolites (ZSM-5, Beta, h-ZSM-5 and h-Beta) were used as heterogeneous catalysts. As expected from the literature, the reaction using homogeneous sulfuric acid gave the highest FAME content in the reaction product (90 wt%). However, the well known disadvantages of homogeneous acid catalysts mentioned in Section 1, made interesting to explore heterogeneous catalysts. Fig. 4 shows the FAME content (expressed as weight percent) in the reaction product obtained over conventional and hierarchical ZSM-5 and Beta zeolites at different temperatures. All the experiments gave a recovered production phase around 50 wt% and, the other phase was the water obtained in the esterification of free fatty acids contained in the microalga (~46 wt%).

Analyzing the effect of reaction temperature, it can be seen how it is necessary to reach 115 °C to reach any activity with ZSM-5 and h-ZSM-5 catalysts. However, Beta and h-Beta zeolites are active catalysts in the esterification of microalga lipids even at 85 °C. The FAME content in the reaction product increases with the temperature being more pronounced for H-Beta zeolite. Standard zeolites presented very low activity, slightly higher for Beta zeolite with higher surface area. FAME production on hierarchical Beta zeolite (h-Beta) was much higher than hZSM-5. The different catalytic behaviors of Beta and ZSM-5 zeolites may be related with their different structures since BEA (for Beta zeolite) is constituted by channel of (7.3 × 6.7) (5.6 × 5.6) Å while MFI (for ZSM-5) has channels of (5.1 × 5.5) (5.6 × 5.3) Å. Therefore, the narrower pore entrance of ZSM-5 zeolite might restrict the diffusion of microalga oil. The differences found between standard and hierarchical zeolites are consequence of the presence of a higher external surface area and, mainly of a considerable amount of supermicropores in the zeolites obtained from silylated seeds. As said before, microporous zeolites show diffusion limitations for the lipids to penetrate to internal acid sites. The development of a great external surface area together with a bimodal porosity in hierarchical zeolites improve the accessibility of bulky lipid molecules to the active sites and therefore, the catalytic properties of these materials.

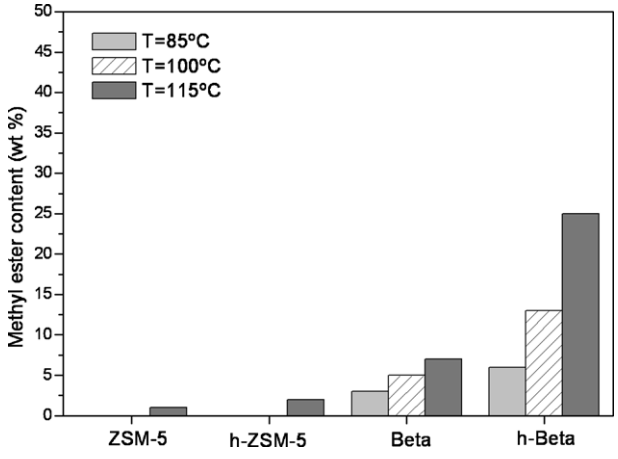

Fig. 4. Comparison of different catalysts and temperatures for FAMEs production.

However, the catalyst acidity may also play a significant role. h-Beta zeolite presents the highest BET area value (725 m^2/g) and high external surface area without losing acidity respect to standard Beta zeolite (~0.40 mmol NH_3/g cat). On the contrary, h-ZSM-5 shows the highest values of external surface (456 m^2/g) but the acid sites population is lower (0.33 mmol/g of acid sites) and consequently this catalyst was almost not active in the biodiesel production under these experimental conditions.

These results indicate that transesterification reaction is very sensitive to changes in porosity, textural properties and acidity of zeolites, which is in agreement with the literature about biodiesel synthesis with solid acid catalysts [27–29].

Finally, it is important to mention that the amount of FAMEs obtained with the best catalyst h-Beta zeolite was not very high but quite promising despite the lower temperatures used in comparison with the literature, for solid acid catalysts, which require higher temperatures and longer reaction times than basic catalyst

[22,23]. Besides, these results could be improved by optimization of the zeolites Si/Al ratio because the Si/Al ratio represents a trade-off between the hydrophobic character and the zeolite acidity. The zeolite must be hydrophobic in order to avoid the absorption of the water by-product that will lead to deactivation. However, if the Si/Al ratio is too high, the zeolite may lose its acidic properties. Nevertheless, at low Si/Al ratio, water is easily adsorbed to the surface, blocking the access of the fatty acids. By increasing the Si/Al ratio, fatty acids can then adsorb to acid sites on the hydrophobic surface and react further (water molecules are unlikely to be adsorbed on sites surrounded by hydrophobic areas) [27].

4. Conclusions

The *N. gaditana* is a suitable microalga to be used as an oil source for biodiesel production. Analyzing the oil extraction process, the highest lipid recovery was reached when the extraction was done with a magnetic stirring under reflux procedure as this method causes significant cell disruption. Moreover, the highest oil content was obtained for the extraction with methanol as solvent (47 wt%), because a polar solvent like methanol may extract better the microalga polar lipids. *N. gaditana* oil contains monounsaturated fatty acids and saturated fatty acids in relatively high concentrations. In fact, microalga oil contained large amounts (around 30%) of palmitic (16:0) and palmitoleic (16:1) acids, together with a 17 wt% of eicosapentaenoic acid (20:5).

Microalga lipids were tested as a feedstock in the biodiesel production using heterogeneous catalysts like hierarchical zeolites, to overcome the disadvantages of homogeneous catalysts currently used in the industrial plants. Analyzing different variables in the biodiesel production, it can be seen how the ester production increased when the temperature increased from 85 °C to 115 °C.

On the other hand, hierarchical h-Beta zeolite catalyst showed the highest activity in the reaction to get FAMEs from *Nannochloropsis* oil. This catalyst presents similar acid strength and number of acid sites than conventional zeolites, with higher external surface area, joined to the presence of a secondary micro-mesoporosity that reduces the diffusion limitations of lipids to reach the zeolite acid active sites.

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