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Effect of Secondary Aldimines on the Oxidation and Microbial Stability of Biodiesel Fuel

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Abstract: *The aim of this study was to investigate the effect of substances with imine functional group on the oxidation and microbial stability of biodiesel fuel. Three secondary aldimines were synthesized and were added at concentrations of 1000, 200 and 50 ppm in two types of fatty acid methyl esters (FAME) from different source material, namely soybean oil and pomace olive oil. Treated FAMEs were also blended with Ultra Low Sulphur Diesel (ULSD) fuel at a concentration of 7% v/v in order to examine the activity of the substances in the final blend. Alterations in the oxidation stability were evaluated by conducting measurements in a Rapid Small Scale Oxidation Test (RSSOT) apparatus. The antimicrobial properties of the aldimines were assessed by employing two different methodologies. At first, the treated FAMEs and their blends were examined by a method detecting the presence of substances that can inhibit the growth of Bacillus stearothermophilus. Secondly, the treated fuel samples were contaminated with bottom water of known viable microbial colonies and these microcosms were stored for a period of 6 weeks. The ability of the examined substances to suppress the microbial activity in the aqueous phase was evaluated by measuring the cellular adenosine triphosphate (cATP) concentration. The results figured that the laboratory formulated aldimines were efficient not only in inhibiting the microbial activity but also in substantially improving the oxidative behavior of the tested fuels particularly in treating rates of 200-1000 ppm. Moreover, these additives were found to be effective in suppressing the microbial growth in contaminated diesel/ biodiesel fuel microcosms. The relative activity of the aldimines seemed to depend on their chemical structure as well as on the type of FAME.*

Keywords: *Aldimines, antimicrobial activity, antioxidant activity, biodiesel, FAME, schiff bases*

I. Introduction

Biodiesel is a renewable fuel, alternative to conventional diesel fuel, consisting of fatty acid methyl or ethyl esters (FAME -FAEE) from vegetable oil or animal fat origin. In spite of the advancements on second and third generation biofuels, biodiesel, and particularly FAME, is still considered universally as one of the most widely produced and employed biofuel [1]. In several countries mandates have been established for its utilization and currently in Europe the maximum allowable concentration of FAME in petroleum diesel amounts to 7% V/V (B7). Biodiesel possesses several advantages such as high biodegradability, low toxicity and alleged benefits on exhaust and greenhouse gas emissions. On the other hand, deterioration issues are the main critical drawbacks of biodiesel. FAME is more prone to oxidative deterioration compared to conventional diesel mainly due to the presence of unsaturated fatty acids in the molecule. The rate of oxidation is proportional to the number of bis-allylic moieties and, thus, poly-unsaturated fatty acids have higher susceptibility than mono-unsaturated ones [2-3]. In order to inhibit the oxidation process and improve the storage stability, the treatment of biodiesel with antioxidant agents is a common practice. Many studies exist in the literature for the effect of various compounds - mainly of phenolic type - on the oxidation stability of biodiesel and its blends [4-6].
Moreover, the chemical composition of FAME along with its hydroscopic nature makes it more "biologically active" and as a result it is more vulnerable to microbiological growth and bio-deterioration. Several in-field incidents and facts from the fuel supply chain indicate that the addition of FAME contributes to the reduced microbial stability of the final fuel blend [7]. Additionally, a series of experimental studies support the susceptibility of biodiesel fuel on microbial growth [7-9]. As a result, the diesel fuel supply chain has to face new challenges associated with microbial contamination symptoms in biodiesel fuel, such as filter clogging, microbial induced corrosion in the infrastructure and sludge formation in storage tanks.

Based on the above, this study was oriented by the idea that since the addition of oxidation inhibitors is a regular practice, substances that could have a dual function, i.e. a simultaneous antioxidant and antimicrobial action, would be beneficial in terms of biodiesel's stability against deterioration. Secondary aldimines is a class of products that could be examined regarding the aforementioned effects. These compounds have the general formula R-CH=N-R´, they are characterized by the presence of imine functional group and are considered as a sub-class of the so-called Schiff base compounds. Schiff bases and their derivatives are widely employed for industrial purposes as catalysts, pigments, polymer stabilizers and corrosion inhibitors. In addition to this, they

are also known for their broad range of activities in biological systems, demonstrating antioxidant, antibacterial and antifungal properties since the nitrogen atom of azomethine has the ability to interfere with normal cell processes [10-12]. In most cases, compounds containing chloro groups have shown increased antimicrobial activity [13]. In spite of being some of the most widely utilized organic compounds, the examination of Schiff base derivatives as additives in renewable substitutes of petroleum is very limited [14]. Therefore, in this work three types of secondary aldimines were synthesized and their antioxidant and antimicrobial activity in biodiesel fuel systems was studied.

II. Experimental

2.1. Materials and Reagents

Pomace Olive Oil (POMO) and Soybean Oil (SBO) were obtained from the local market and were used without further purification. Table 1 presents the physicochemical properties of the oils engaged in FAME production. Methanol, 99.99% purity was obtained from Fisher Scientific and Sodium Methoxide, pure, anhydrous powder, was taken from Acros Organics. Extra pure β-Aminoisobutyl alcohol, 5-Chlorosalicylaldehyde, 2,4-Dihydroxybenzaldehyde, Epichlorohydrin, Ethanol, 2-Hydroxybenzylamine and o-Phenylenediamine were taken from Sigma-Aldrich.

Table 1. Physicochemical properties of the parent oils

Property	Units	**POMO**	**SBO**	Standard Method
Density @ 15°C	kg/m^3	**918.3**	**923.1**	EN ISO 12185
K. Viscosity @ 40°C	mm^2/s	**40.27**	**32.87**	EN ISO 3104
Water Content	mg/kg	**300**	**300**	EN ISO 12937
Acid Value	mg KOH/g	**0.35**	**0.30**	EN 14104
Saponification Value	mg KOH/g	**195**	**201**	AOAC CD3-25

2.2. Preparation of Fatty Acid Methyl Esters

The corresponding fatty acid methyl esters - POME and SBOME - were produced from the previously mentioned parent oils by alkaline transesterification reaction. Methanolysis was carried out for 2h in a 2L flask at 65^o C using 500g of oil and a 6:1 methanol/oil molar ratio. Sodium methoxide (CH_3ONa) was employed as catalyst at a concentration of 0.75 wt%. After the completion of the transesterification, the upper methyl esters phase was separated from the glycerol phase and was purified by washing with 5% w/w sulfuric acid (H_2SO_4) followed by warm water. The excess of methanol was removed by rotary evaporator. The purified methyl esters were dried over anhydrous sodium sulphate (Na_2SO_4) and after vacuum filtration the final FAMEs were obtained. The quality parameters of the produced methyl esters are given in Table 2 and were analyzed according to the applicable requirements and test methods indicated by the European Standard EN14214:2012.

Table 2. Quality parameters of the prepared FAMEs

Property	Units	**POME**	**SBOME**	EN14214 Limits	Standard Method
Ester Content	% m/m	**97.8**	**98.6**	min 96.5	EN 14103
Density @ 15°C	kg/m^3	**880.3**	**885.2**	860-900	EN ISO 12185
Kin. Viscosity @ 40°C	mm^2/s	**4.814**	**4.133**	3.50-5.00	EN ISO 3104
Oxidation Stability, (RSSOT)	minute	**35**	**18**	-	EN 16091
Linolenic acid methylesters content	% m/m	**0.68**	**5.14**	max 12.0	EN 14103
Water Content	mg/kg	**200**	**200**	max 500	EN ISO 12937
Acid Value	mg KOH/g	**0.28**	**0.21**	max 0.50	EN 14104
Iodine value	g I_2/100g	**83**	**125**	max 120	EN 14111
Monoglyceride content	% m/m	**0.27**	**0.22**	max 0.70	EN 14105
Diglyceride content	% m/m	**0.14**	**0.05**	max 0.20	EN 14105
Triglyceride content	% m/m	**0.07**	**0.08**	max 0.20	EN 14105
Free glycerol	% m/m	**<0.01**	**<0.01**	max 0.02	EN 14106
Total glycerol	% m/m	**0.10**	**0.08**	max 0.25	EN 14105

Their fatty acid composition as listed in Table 3 was determined by gas chromatography using a DANI Master GC instrument in accordance with EN14103. POME is abundant in oleic acid (C18:1), whereas SBOME consists predominantly of linoleic acid (C18:2). Considerably higher levels of linolenic acid (C18:3) were detected in SBOME.

Table 3. Fatty acid composition of the prepared FAMEs

Fatty acid profile		% wt. content	
		POME	SBOME
Palmitic	C16:0	10.77	10.29
Palmitoleic	C16:1	0.78	0.10
Stearic	C18:0	2.73	4.24
Oleic	C18:1	72.33	24.11
Linoleic	C18:2	10.47	52.64
Linolenic	C18:3	0.68	5.14
Arachidic	C20:0	0.58	0.47
Gadoleic	C20:1	0.54	0.28
Behenic	C22:0	0.28	0.54

2.3. Preparation of B7 Blends

FAME samples were blended with an ultra-low sulphur diesel (ULSD) at a concentration of 7% V/V (B7), equal to the maximum allowable mixing ratio according to EN590:2013. The ULSD base fuel sample was a hydrotreated atmospheric straight run gasoil, it was supplied from a local refinery and it was additive-free. All the prepared blends were examined regarding their basic physicochemical properties as listed in Table 4.

Table 4. Quality parameters of the B7 Blends

Property	Units	**B7 POME**	**B7 SBOME**	EN 590 Limits	Standard method
Kin. Viscosity @ 40°C	mm^2/s	**3.770**	**3.776**	2.00-4.50	EN 3104
Density @ 15 °C	kg/m^3	**839.3**	**839.6**	820-845	EN 3675
Sulphur content	mg/kg	**8.6**	**8.5**	max 10.0	EN 20846
Water Content	mg/kg	**100**	**100**	max 200	EN 12937
Acid Value	mg KOH/g	**0.15**	**0.14**	-	ISO 6618
Oxidation Stability, (RSSOT)	minute	**88**	**45**	-	EN 16091
FAME Content	% (V/V)	**7**	**7**	max 7.0	EN 14078

2.4. Synthesis of the secondary aldimine additives

The three secondary aldimines (A,B,C) were synthesized in the laboratory, according to the general scheme shown in Fig. 1, by reacting 5-chlorosalicylaldehyde with o-phenylenediamine for compound A, while for the other two compounds (B & C) an initial reaction of 2,4-dihydroxybenzaldehyde with epichlorohydrin was followed by a second reaction of the intermediate product with either 2-hydroxybenzylamine or β-aminoisobutyl alcohol for obtaining aldimines B and C, respectively. In order to acquire the desired products, the reaction parameters and purification steps were applied as per literature [15-17]. Their nomenclature is listed in Table 5 along with some of their characteristics. Chemical compositions analysis (C/H/N/O/Cl) was performed in a multiEA5000 (AnalyticJena) and a EuroEA3000 (Eurovector) elemental analyzer. All substances belong to the family of Schiff base compounds and contain chloro moieties in their molecule. Substance A has a higher content in nitrogen and chlorine due to the presence of two imine functional groups and two chloro-groups. Substances B and C have a similar mono-imine structure but they are differentiated in the R' position (aromatic for B, aliphatic for C). These substances were tested for their effectiveness as antioxidant and antimicrobial agents in biodiesel fuel. In order to facilitate the mixing of these additives with the produced methylesters, they were pre-dissolved in acetone at a concentration of 30% m/m and afterwards they were added in FAMEs in this form followed by complete evaporation of the solvent.

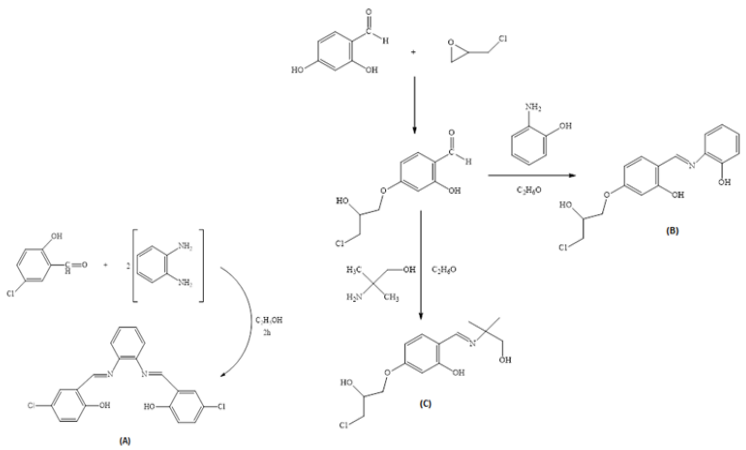


Figure 1. Simplified pathway for producing the secondary aldimines A, B and C.

Table 5. Nomenclature and properties of the synthesized secondary aldimines

Code	Nomenclature	Chemical Formula	M.W.	M.P. (°C)	Elemental composition (% m/m)				
					C	H	N	O	Cl
A	N,N'-bis(5-chloro-2-hydroxybenzylaldehyde)1,2-phenylene diimine	$C_{20}H_{14}(ClNO)_2$	385.2	154	62.1	3.6	7.2	8.2	17.9
B	5-(3-chloro-2-hydroxypropoxy)-2-{(E)-[(2-hydroxyphenyl)imine] methyl} phenol	$C_{16}H_{16}ClNO_4$	321.8	166	59.2	4.9	4.3	19.6	10.8
C	5-(3-chloro-2-hydroxypropoxy)-2-{(E)-[(2-hydroxy-1,1-dimethyl-ethyl)imine]methyl} phenol	$C_{14}H_{20}ClNO_4$	301.8	142	55.5	6.8	4.5	21.0	11.2

2.5. Methodology

The three aldimines were added separately to each FAME at a treating rate of 0.1% m/m (1000 ppm), 0.02% m/m (200 ppm) and 0.005% m/m (50 ppm) in order to assess their effectiveness regarding the oxidation and microbial stability in a variety of concentrations. Moreover, both FAME samples treated with the three additives at 1000 ppm were blended with ULSD at a mixing ratio of 7% V/V (B7), and the concentration of the additives in the final blends was calculated to be equal to about 75 ppm.

2.5.1. Oxidation stability measurements

The effect of the examined aldimines on biodiesel's relative resistance to degradation, was assessed under conditions of high pressure and temperature in the Rapid Small Scale Oxidation Test (RSSOT- PetroOxy, Petrotest) according to the ASTM D 7545/ EN 16091 Standard method. A 5ml sample was introduced into the pressure vessel of the apparatus which was then charged with oxygen to 700 kPa at ambient temperature. The test was initiated by rapidly heating the pressure vessel to the selected temperature of 140 ^{o}C. The pressure in the test apparatus was recorded continuously until it was 10 % below the maximum pressure of the actual test run (break point). The lapsed time between starting the heating procedure of the sample vessel and the break point is reported as Induction Period (IP) in minutes. A typical graph showing the concept of RSSOT determinations is given in Fig. 2.

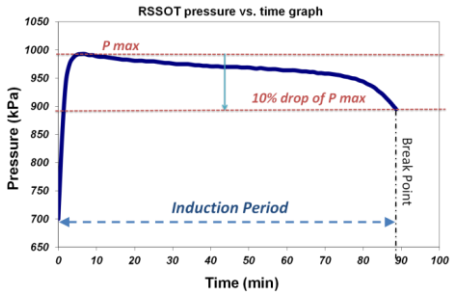


Figure 2. Typical pressure vs. time RSSOT graph

2.5.2. Microbial stability assessment

The antimicrobial properties of the secondary aldimines were assessed by employing two methodologies.

2.5.2.1. Screening the antimicrobial potential of aldimines in biodiesel

The evaluation of the ability of these additives, in the selected concentrations, to inhibit the microbial growth, was performed by employing commercially available ampoules of gel comprising of solid agar, nutrients, a colour indicator and spores of *Bacillus stearothermophilus* - a gram-positive bacterium (Biocide Rapide Test - Echa Microbiology). A small quantity of the fuel to be tested was added to the ampoule which was then incubated at 64°C for 3.5 hours. The inhibitory potential of the sample was evaluated by examining the colour of the gel in the ampoule at the end of the test. As shown in Fig. 3, no change in the color (purple) depicts the ability of the sample to suppress the bacillus' growth, whereas a change in the color (yellow) is indicative of the non-inhibitory activity of the tested sample.

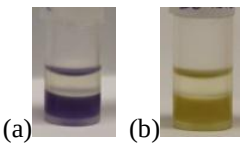


Figure 3. Typical interpretation for evaluating the antimicrobial potential in the fuel samples. (a) Inhibition, (b) no inhibition.

2.5.2.2. Antimicrobial activity of aldimines in challenged microcosms

In the second phase of the study, the ability of the examined aldimines to suppress already existing heavy microbial load was investigated, in laboratory-scale microcosms simulating short-term storage systems and conditions in the diesel/biodiesel fuel supply chain. Taking into consideration that in such cases usually a so-called shock dose treatment of a biocide compound is employed, the effect of the maximum previously examined concentration of aldimines was studied i.e. 0.1% w/w. In this part of the study only POME was employed because of its higher storage stability compared to SBOME. Microcosms consisting of treated POME with 1000ppm of each additive and B7 POME-ULSD blends with 75ppm of each additive were set up in duplicate and were inoculated (at a 3:1 fuel-water ratio) with a contaminated "bottoms-water" comprising of an uncharacterized inoculum of known initial microbial activity. This challenge inoculum was prepared in accordance to ASTM E1259 standard method from the aqueous phase of a microbially contaminated commercial diesel fuel from retail station tanks. The microcosms were stored in sterile containers for a period of 6 weeks. For comparison reasons, microcosms of untreated POME and B7 blend were also inoculated and stored for an equal period and under the same ambient - low light conditions. The alterations in the microbial proliferation in the microcosms were evaluated by collecting and analyzing samples from the aqueous phases at predetermined intervals, namely after 2 and 6 weeks of storage time.

The microbial activity of the aqueous phases was monitored by carrying out measurements according to the ASTM D7687-11 Standard Test Method for Measurement of Cellular Adenosine Triphosphate (cATP) in Fuel, Fuel/Water Mixtures, and Fuel-Associated Water with Sample Concentration by Filtration. This test method measures the concentration of cATP - a constituent of all living cells including bacteria and fungi. The presence of cellular-ATP is an indicator of total metabolically active microbial contamination and reflects the total bioburden in the sample. The collected samples were filtered and washed with the designated agents so as to extract the ATP. The latter was measured using a bioluminescence enzyme assay, whereby light was generated in amounts proportional to the concentration of cATP in the samples. By calculating the intensity of the emitted light (as Relative Light Units - RLU), the ATP concentration was quantified and the total bioburden was reported as pg ATP/ml. The ATP test is a direct and relatively rapid microbiological test method compared to culture methods. All oxidation and microbial stability tests were performed in duplicate in order to verify the repeatability of the determinations and the mean value of the measurements is reported along with the precision.

III. Results and Discussion

3.1. FAME acidity

Initially, the effect of the examined aldimines on the acidity of FAMEs was examined. Acid value determinations were conducted per EN 14104 standard method in the treated POME and SBOME samples. As shown in Table 6, under the highest treating rate of 1000ppm, the addition of the three aldimines results in a slight increase in FAME's acidity, however in all cases the values are below the maximum of 0.5 mg KOH/g designated in EN14214 biodiesel specifications.

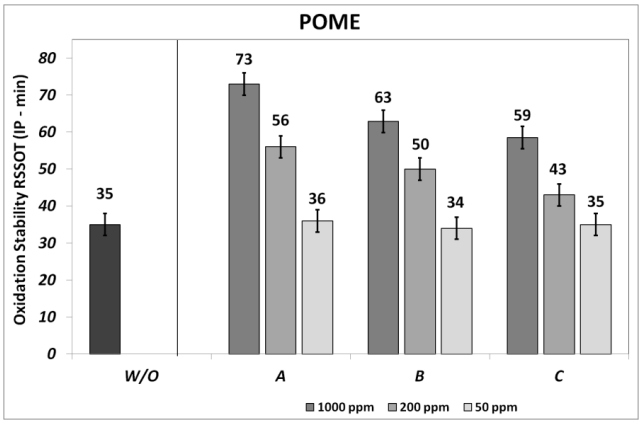
Table 6. Alterations in the Acid Value of the prepared FAMEs after the addition of the three aldimines at a concentration of 0.1% w/w (1000 ppm)

Additive	Acid Value (mg KOH/g)	
	POME	SBOME
A	0.42	0.40
B	0.38	0.35
C	0.33	0.28

3.2. Oxidation Stability

Fig. 4 presents the results obtained from the oxidation stability measurements in the RSSOT before (w/o) and after the addition of the three examined aldimines (A, B & C) in neat POME and SBOME. The higher oxidation stability of the untreated POME is attributed to the higher content of mono-unsaturated fatty acids compared to the poly-unsaturated character of SBOME. Regarding POME, all tested substances demonstrated a positive effect on the induction period when added at concentrations of 1000 and 200 ppm, whereas, at the lowest treating rate of 50 ppm, they were not capable of substantially upgrading the aging reserve of the fuel. Substance A proved to be the most efficient in improving the oxidation stability of POME and significantly increased the induction period especially at the treating ratio of 1000 ppm. The antioxidant activity of the tested compounds was found to be in the order of A > B > C. In SBOME, the addition of the aldimines at 1000 ppm also enhanced FAME's oxidation stability but contrary to the behavior that was observed in POME, the antioxidant efficiency was more or less equivalent for all three substances. Compared to published data on SBOME, all aldimines appear to have a similar activity with BHT - a commonly used inhibitor - at 1000 ppm [4]. Lower treating rates of 200ppm and 50 ppm resulted in either a slight or negligible improvement of SBOME's oxidation characteristics.

Taking into consideration the relative increase in the induction periods, it is obvious that the tested secondary adimines were more capable antioxidants in the case of the monounsaturated POME compared to the polyunsaturated SBOME. In the B7 blends, the synthesized aldimines gave rise to a considerable improvement of the oxidative characteristics as shown in the results given in Fig. 5. Particularly, the addition of A increases the oxidation stability of B7 POME by 165%. When comparing the percent increase in the induction periods between pure FAMEs and their corresponding blends it is evident that in most cases the tested aldimines show a stronger activity in the B7 blends. This could imply that the presence of these imine-type substances might enforce the oxidation stability not only of FAME but also of the conventional ULSD. Nevertheless, it appears that the relative activity observed in the neat FAMEs is imparted in the B7 blends as well, i.e. in the POME blends the relative activity was A>B>C whereas in the SBOME blends the addition resulted in more or less similar increase in the induction period.



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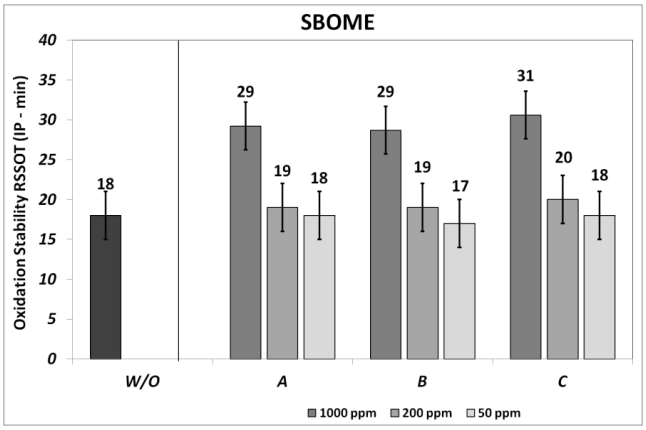


(b)

Figure 4. Effect of the synthesized aldimines on the oxidation stability of (a) POME and (b) SBOME

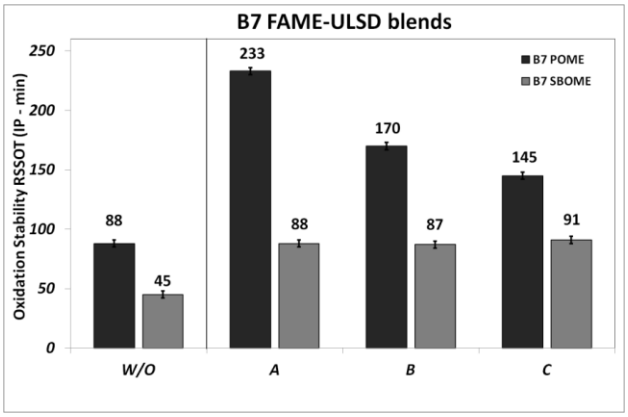


Figure 5. Effect of the synthesized aldimines on the oxidation stability of B7 blends (A,B & C were added at 0.1% w/w in the neat FAMEs before blending with ULSD)

3.3. Microbial Stability

3.3.1. Antimicrobial potential of aldimines in biodiesel

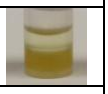
The results from the initial screening of the three aldimines, regarding their antimicrobial behavior when added in various concentrations in the two types of FAME (POME and SBOME), are presented in Table 7 accompanied by the respective pictures of each ampoule at the end of the test. The symbol "+" indicates an inhibitory effect in the growth of the Bacillus, whereas the symbol "-" means that the examined sample is incapable of suppressing the bacterial growth or that it may contribute to its proliferation. In order to verify that the test could give a sound indication of the antimicrobial properties of the additives, at first the non-treated FAMEs were incubated and, as expected, in both cases the ability of the thermophilic bacillus to grow was observed.

Table 7. Ability of FAMEs to inhibit the growth of *Bacillus stearothermophilus* when treated with the synthesized aldimines at 1000, 200 and 50 ppm

FAME	w/o	**A**			**B**			**C**		
		1000	*200*	*50*	*1000*	*200*	*50*	*1000*	*200*	*50*
POME	-	+	+	+	+	+	+	+	+	-
										
SBOME	-	+	+	-	+	+	-	+	+	-
										

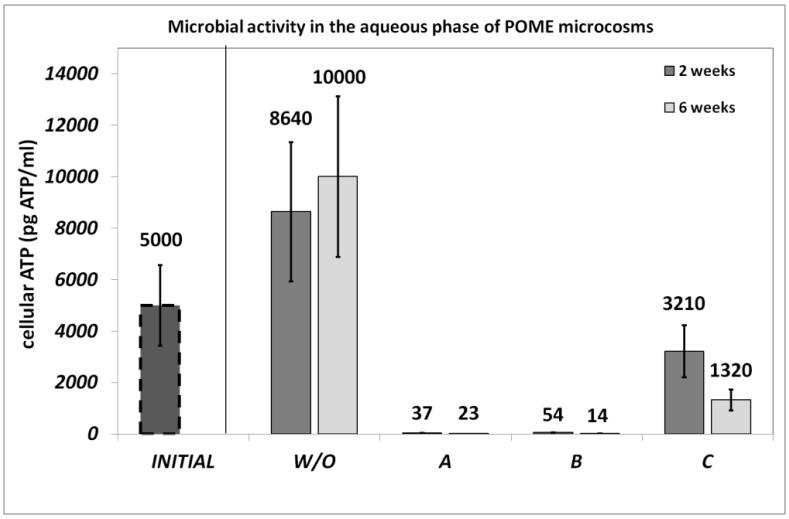
After the addition of the three synthesized aldimines at concentrations of 1000 ppm and 200 ppm, both FAMEs demonstrated strong antimicrobial activity against the growth of the Bacillus. At the rate of 50 ppm, the presence of additives A and B was still beneficial for the microbial stability of POME, however in the case of SBOME, they were not so efficient in inhibiting the bacterial growth. Regarding substance C, it was incapable of substantially suppressing the microbial proliferation when added at the lowest tested treating rate. The antimicrobial effect of the three aldimines against *Bacillus stearothermophilus* was also assessed in the B7 FAME-ULSD blends and the results are listed in Table 8. As previously mentioned, FAMEs were treated with 1000ppm of each additive, therefore, a concentration of about 75ppm was present in the final blend. Again the untreated B7 fuels (w/o) were tested in the beginning and showed no inhibiting activity. Of the treated B7 blends, those that contained the A and B additives depicted an antimicrobial behavior. On the contrary, the presence of C at this ratio could not adequately prohibit the bacillus' growth.

Table 8. Ability of B7 blends to inhibit the growth of *Bacillus stearothermophilus* when treated with the synthesized aldimines.

FAME/ULSD blend	w/o	**A**	**B**	**C**
B7 POME	-	+	+	-
				
B7 SBOME	-	+	+	-
				

3.3.2. Antimicrobial activity of aldimines in challenged microcosms

In the second phase of the antimicrobial study, there was an attempt to evaluate whether the synthesized aldimines were able to either suppress or eliminate the proliferation of an active bottoms-water bioburden in the laboratory-scale biodiesel microcosms. Taking into account the previously presented results on the initial antimicrobial screening, the array of the POME microcosms included all three additives (A,B & C), whereas in the B7-POME/ULSD microcosm only A and B aldimines were employed, since C showed no antimicrobial effect in B7 samples. Just before inoculation, the microbial activity of the bottoms-water/inoculum was determined and found to be equal to 5000 pg ATP /ml. The results obtained from the two arrays of microcosms during the monitoring period of 6 weeks are presented in Fig. 6.



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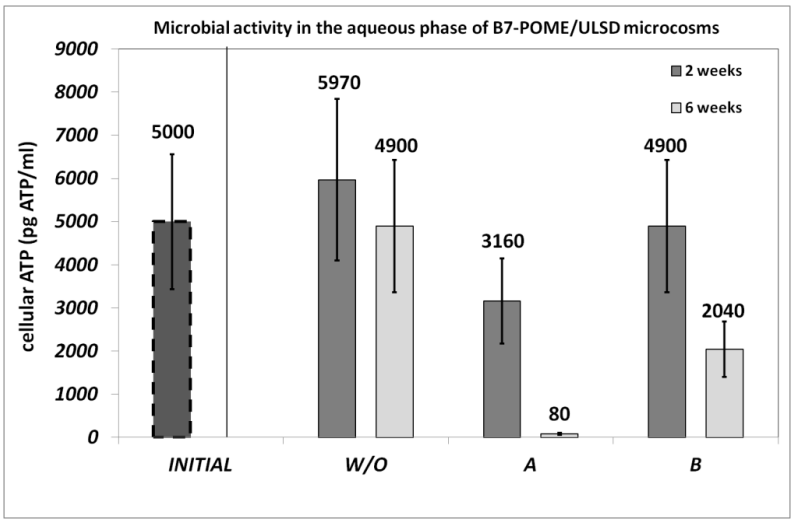

(b)

Figure 6. Effect of the synthesized aldimines on the microbial activity in the aqueous phase of (a) POME microcosms, (b) B7 POME/ULSD microcosms (treatment with 1000 ppm on FAME basis)

After 2 weeks of storage, the microbial activity in the aqueous phase of the treated POME microcosms was significantly suppressed - especially in those containing the aldimines A and B. In particular, the presence of compounds A and B resulted in almost total elimination of the active bioburden, since the measured ATP concentration was reduced by 99% compared to the initial. A lower, but still noticeable, reduction of 36% in the microbial load was observed in the POME microcosm containing the C substance. The antimicrobial activity of the synthesized aldimines is also highlighted by the fact that in the untreated POME microcosm the ATP concentration was substantially increased from 5000 to 8640 pg ATP/ml, a sign indicative of the susceptibility of FAMEs on microbial proliferation. In the case of B7 samples, a 37% and 2% reduction in the ATP concentration compared to the initial microbial load was observed in the microcosms treated with A and B substances, respectively. Though these values appear unequal to those demonstrated in the treated POME microcosm, the much lower aldimine concentration in the blends should be taken into consideration. In this context, the antimicrobial efficiency of substance A is still notable and comparable to the activity of the much higher concentration of substance C in the POME microcosm. Besides, after 2 weeks the aqueous phase's bioburden under the untreated B7 blend was increased by 20%.

After 6 weeks of storage, the microbial activity in the bottoms-water of POME with aldimines A and B was practically eradicated, while in the C-treated microcosm the ATP concentration was further reduced by 60% but not as drastically as in the case of A and B. On the contrary, in the untreated POME microcosm the bioburden proliferation continued and a twofold increase was reported compared to the initial. In B7 samples, the ATP concentration in the presence of substance B was further decreased and was found to be less than half of the initial bioburden after 6 weeks. However, substance A proved to be a much more efficient antimicrobial

additive in the B7 microcosms. The microorganisms' activity in the aqueous phase was strongly suppressed and at the end of the monitoring period negligible bioburden of 80 pg ATP/ml was detected. In a nutshell, from the experiments on the antimicrobial behavior of the synthesized aldimines in biodiesel fuel, it was found that all tested substances could inhibit microbial growth especially when added at concentrations of 200ppm and higher. However, substances A and B proved to be more efficient in enhancing the microbial stability of biodiesel fuel compared to substance C. Not only they exhibited antimicrobial activity in POME at concentrations lower than 200ppm, but also they significantly suppressed microbial growth in the microcosms' experiments. Besides, substance A, was the most capable aldimine in the case of B7 blends microcosms. By focusing on the structure-activity relationship, these results imply that heteroatoms, such as nitrogen, and the aromaticity might play an important role in the enhancement of the microbial stability of biodiesel fuel systems. The activity was increased in the presence of a second imine functional group as well as in those substances with higher aromaticity. In the case of A, the existence of a second chloro-group might have also been beneficial, however when comparing the antimicrobial activity of B and C it was shown that higher content of aromatic moieties resulted in improved performance, irrespective of the presence of a chloro-moiety. Generally, the above observations are in line with what is reported in the literature regarding the parameters that affect the antimicrobial activity of Schiff base derivatives in biological systems [10-13].

IV. Conclusion

In this study two types of FAME (POME & SBOME) and the corresponding B7 Blends were treated with three laboratory-synthesized secondary aldimines in order to evaluate the latter's effect on the oxidation and microbial stability of biodiesel fuel. The antioxidant activity was measured according to the RSSOT method, whereas the antimicrobial efficiency was assessed by examining the inhibitory potential against the growth of a certain bacillus and by monitoring the alterations in the ATP concentration in the aqueous phase of previously contaminated microcosms. The results can be interpreted as follows. The three synthesized aldimines were capable of improving both the oxidation and the microbial stability of FAMEs, especially at the treating rates of 1000 and 200 ppm. No adverse effect on the acidity was observed. In the B7 blends, the presence of aldimines at a concentration of 75 ppm (1000ppm in FAME) substantially enhanced the aging reserve of the fuel, however regarding the antimicrobial properties only two of the examined substances exhibited inhibitory behavior. Of the three tested aldimines, the one with a di-imine chemistry - containing three aromatic rings and two chloro-groups - overall demonstrated the best performance, followed by the mono-imine substance with increased aromaticity. It appears that the higher aromaticity can have a positive effect in the behavior of the aldimines in biodiesel fuel systems. The fatty acid profile of FAME also plays a role on the relative activity of aldimines with them being generally more beneficial to the largely mono-unsaturated POME. Overall, it appears that secondary aldimines, and Schiff base derivatives as a whole, could be an interesting class of products to be considered as dual function antioxidant and antimicrobial additives in biodiesel fuel. Further examination could focus on the structure-activity relationships of aldimines in biodiesel fuel systems and indicate those substances with an optimum performance per type of FAME.

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Abbreviations

ATP	Adenosine Triphosphate
B7	Diesel/Biodiesel blends with 7% V/V FAME
BHT	Butylated Hydroxytoluene
FAME	Fatty Acid Methyl Esters
IP	Induction Period
M.P.	Melting Point
M.W.	Molecular Weight
POME	Pomace Olive Oil Methyl Esters
POMO	Pomace Olive Oil
RSSOT	Rapid Small Scale Oxidation Test
SBO	Soybean Oil
SBOME	Soybean Oil Methyl Esters
ULSD	Ultra Low Suphur Diesel

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