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Microbial diversity in trace water of jet fuel in Vietnam

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Introduction

Microorganisms in petroleum product, including jet fuel, have been found by some English and American authors since the 20's of the last century. In 1958, after Bakanaukas isolated many microorganisms in jet fuel JP4, this topic was especially concentrated. Most of microorganisms living in jet fuel are able to degrade hydrocarbon of the fuel to obtain energy and carbon source for their growth. The presence of microorganisms in jet fuel not only brings about the lost of fuel in store process, but also briefly changes the component and structure of jet fuel. More over, the products of their metabolism, such as organic acids, alcohols, H_2S... are corrosive. Hence, they cause many problems for fuel reservoirs and tanks, leak plugging pipelines. Besides, filter system and plugging pipeline can become obstructed because of microorganisms' biomass. This influences the plane safety. Therefore, many countries in the world consider microorganism criteria as an important standard to estimate the jet fuel quality.

From 1985 to 1990, in our country, Vietnam, there were some science projects about microorganisms in TC1 fuel. At the present, as all country in Asia, Vietnam used to utilize and import hundred tons of jet A1. Tropical climate of Vietnam is the suitable condition for growing of microorganisms. With C_8 - C_{18} alkane chains, jet fuel is carbon source for a lot of microorganisms, especially bacteria and fungi, which harmfully affect plane safety. Therefore, we aimed Vietnam airlines company to control the number of microorganisms in jet fuel of all Vietnam airplanes, as well as study on the diversity of microorganisms in trace water in fuel samples, looking for the reason why filter is obstructed to contribute to the jet fuel storing and safety in Vietnam.

Materials and methods

- Collect JetA1 samples from two water valves in two airplane wings.

- Inoculate, isolate and estimate the number of microorganisms in selective media: R_2A and MPA for bacteria, Czapeck for fungi, Hansen for yeast, Gause for actinomyces, Gost -902374 for jet A1 degrading bacteria.

- Observe microorganism cell morphology then photograph microorganisms under Japanese JEM 1010 electromicroscopy.

- Identify some common species in JetA1 with biochemical tests of Biomerieux (API20NE, API50CHB, API 50CH)

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- Study on the diversity of microorganism population by 16S rDNA analysis and DGGE method.

- Estimate the chemical and biological changes of jet fuel affected by microorganisms with content method and gas-chromatography.

- Treat the data with Pros Nelson 1987.

- Identify chemical and biological component of jet fuel filter in several planes to estimate the capacity of making obstructed filter of microorganisms.

Results and discussion

The results involved in three main parts:

A. Analyzing the number of microorganisms in trace water and fuel samples collecting from airplane at the time they land off.

B. Estimating the influence of microorganisms on chemical component of fuel.

C. Analyzing the microorganisms and chemical structure of fuel filter samples.

A. Analyzing the number of microorganisms in trace water and fuel samples collecting from airplane at the time they land off

1. The number of microorganisms in fuel trace water samples from airplanes

Table 1. The number of microorganisms in fuel trace water samples from airplanes of Vietnam airlines

Sample on	Sample	The number of microorganism (CFU/ml)		
		Aerobic bacteria	fungi	JetA1 degrader
30.10.02	S7 - ASA	1.2×10^7	$< 10^1$	$<10^1$
10.3.03	S7 - ASA	7.6×10^6	0	$<10^1$
7.3.03	S7 - ASB	5×10^4	$< 10^1$	$<10^1$
28.4.03	S7 - ASC	1.5×10^7	1.6×10^2	$<10^1$
4.12.02	S7 - ASD	4.7×10^6	$\mathbf{6.6 \times 10^5}$	$<10^1$
11.4.03	S7 - ASD	10^6	$< 10^1$	$<10^1$
20.1.03	S7 - ASE	6.1×10^5	10^1	$<10^1$
10.6.03	S7 - ASE	2×10^7	3.5×10^1	$<10^1$
10.1.03	S7 - ASF	6.6×10^6	1.5×10^1	0
26.5.03	S7 - ASF	$\mathbf{1.2 \times 10^8}$	$\mathbf{5 \times 10^3}$	$<10^1$
13.2.03	S7 - ASG	2.4×10^7	0	$<10^1$
21.10.02	S7 - ASH	$\mathbf{10^1}$	$\mathbf{8.8 \times 10^3}$	$<10^1$
23.1.03	S7 - ASH	1.8×10^4	$< 10^1$	$<10^1$
9.12.02	S7 - ASI	8.4×10^5	2.8×10^3	$<10^1$
16.1.03	S7 - ASI	2.4×10^5	$< 10^1$	0
14.4.03	S7 - ASI	5.6×10^7	$< 10^1$	$<10^1$
23.12.02	A321 - 342	2×10^6	10^3	$<10^1$
2.6.03	A321 - 342	4.4×10^7	0	0
21.10.02	A321 - 346	2.4×10^6	2.8×10^1	$<10^1$
5.3.03	A321 - 346	1.3×10^6	$< 10^1$	$<10^1$
3.4.03	A321 - 346	4×10^6	10^1	$<10^1$
23.1.03	F70 - 502	2.5×10^3	0	$<10^1$
21.1.03	F70 - 504	10^6	2.5×10^1	10^1
21.10.02	767 - A769	7.5×10^4	0	$<10^1$

In all 24 of trace water samples in jet fuel microorganisms are present. The minimum number of microorganisms is 10^4 CFU/ml (S7-ASH on 10.21st.02) and the maximum number is 10^8 CFU/ml (S7-ASF on 5.26th.03 with 10^8 bacteria/ml and 10^3 fungi/ml).

Fungi are present in 19/24 analyzed samples and the maximum number of fungi is 6.6 x 10^5 CFU/ml in S7-ASD sample on 12.4th.02. Only 5 samples have no fungi.

JetA1 degrading microorganisms are present in 21/24 samples. In some samples, there are both fungi and JetA1 degrading bacteria (S7-ASD, 12.4th.02; A321-346, 10.21st.03). Only in 3 samples, there are no JetA1 utilizing microorganisms.

In an airplane but in different times of sample collecting, the number of microorganisms is different, depending on: the collecting time, flight time and biocide treating time.

2. The number of microorganisms in jet fuel samples

Table 2. The microorganism density in JetA1 fuel from planes of Vietnam airlines

Day	Samples	Density of microorganism (CFU/ml)				
		Bacteria	Fungi	Yeast	Actino-myces	Jet A1 degrader
30.10.02	S7 - ASA	$<10^1$	$<10^1$	0	0	$<10^1$
10.3.03	S7 - ASA	3×10^1	0	0	0	$<10^1$
7.3.03	S7 - ASB	$<10^1$	$<10^1$	0	0	$<10^1$
28.4.03	S7 - ASC	1.5×10^1	4.4×10^1	0	0	$<10^1$
4.12.02	S7 - ASD	1.9×10^2	2.3×10^1	0	0	$<10^1$
11.4.03	S7 - ASD	4×10^1	$<10^1$	0	0	$<10^1$
20.1.03	S7 - ASE	7.5×10^1	10^1	0	0	$<10^1$
10.6.03	S7 - ASE	$<10^1$	3.5×10^1	0	0	$<10^1$
10.1.03	S7 - ASE	1.5×10^2	1.5×10^1	0	0	0
26.5.03	S7 - ASF	4×10^1	1.1×10^3	0	$<10^1$	$<10^1$
13.2.03	S7 - ASG	6×10^1	0	0	0	0
21.10.02	S7 - ASH	$<10^1$	1.3×10^1	0	0	$<10^1$
23.1.03	S7 - ASH	$<10^1$	$<10^1$	0	0	$<10^1$
9.12.02	S7 - ASI	$<10^1$	$<10^1$	0	0	$<10^1$
16.1.03	S7 - ASI	0	$<10^1$	0	0	0
14.4.03	S7 - ASI	4.5×10^1	$<10^1$	0	0	$<10^1$
23.12.02	A321 - 342	$<10^1$	0	0	0	$<10^1$
2.6.03	A321 - 342	$<10^1$	0	0	0	0
21.10.02	A321 - 346	1.7×10^2	1.7×10^1	0	0	$<10^1$
5.3.03	A321 - 346	8×10^2	$<10^1$	0	0	$<10^1$
3.4.03	A321 - 346	2×10^1	10^1	0	0	$<10^1$
23.1.03	F70 - 502	1.3×10^1	0	0	0	$<10^1$
21.1.03	F70 - 504	2.8×10^2	2.5×10^1	0	0	10^1
21.10.02	767 - A769	1.7×10^2	1.7×10^1	0	0	$<10^1$

According to the table 1 and 2, microorganisms in JetA1 fuel in Vietnam are diverse with all representation: aerobic bacteria, JetA1 utilizing bacteria, yeast, actinomyces and fungi. Although collecting sample at the same time and place, the number of microorganisms in samples with trace water is thousand times higher than that in fuel samples. This means that it is necessary to control and drain water in reservoirs frequently, associate storing process with treating fuel filter during supplying plane. As a result, we will decrease the number of microorganisms markedly.

3. DGGE analysis of bacterial population in jet fuel taken from airplanes

After isolation crude DNA of total population in jet fuel we carried out DGGE analysis (figure 1).

There are many bands in some column. The results of DGGE were very suitable to analyzed data on selective media (table 1 and 2). In the next step, we should cut these bands for further analysis.

Basing on morphology and biophysical-biochemical characteristics, as well as biochemical tests and 16S rDNA, we classified some common species in JetA1 fuel.

Figure 1. DGGE of contaminents in jet fuel

1, 2: ASA
3, 4: ASB
5, 6: ASI
7, 8: A346
9, 10: A769

Gram-negative bacteria involved in *Aeromonas, Chryseomonas, Pseudomonas (P. aeruginosa, P. putida, P. fluorescens, P. cepacia)*

Gram-positive bacteria involved in *Bacillus (B. coagulans, B. ciculans), Mycobacterium, Micrococcus*

Fungi involved in *Aspergillus (A. viridi, A .mutans, A. versicolor, A. sydovy, A. flavipes), Aureobasidium, Acremonium bacillispirilum, Paccillomyces variotii, Penicillium (P. lilacium, P. pullularia, P. nigricans, P. citrinum)*

Yeast involved in *Candida (C. tropicalis, C. albicans), Rhodoturula (R. glutinis)*

Actinomyces involved in *Streptomyces (S. argenteolus, S. orientalis, S. pulcher)*

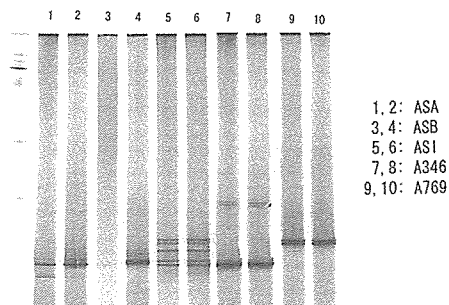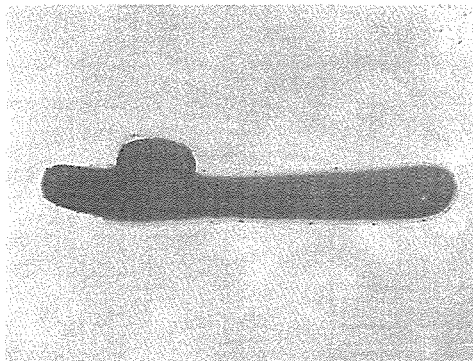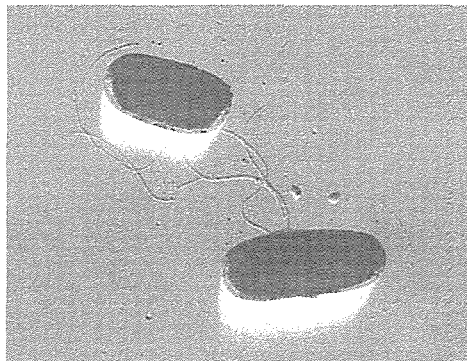*Bacillus coagulans ASB*   *Chryseomonas ATR- KOC*

Figure 2. The morphology of *Bacillus coagulans ASB* and *Chryseomonas ATR- KOC* viewed with the electromicroscopy

4. The number of microorganisms in fuel samples from reservoir and filter

Table 3. The ratio of microorganism groups in analyzed samples from reservoir and filter

The number of microorganism (CFU/ml)	The number of samples with microorganisms					
	Aerobic bacteria	Anaerobic bacteria	JetA1 degrading microorganism	Actino-myces	Fungi	Yeast
$> 10^4$	25	0	2	0	1	0
$10^2 - 10^4$	46	1	34	5	20	3
$10^1 - 10^2$	48	5	43	8	67	7
$<10^1$	119	6	78	13	88	10
Total	**238**	**12**	**158**	**26**	**176**	**20**

Table 4. The ratio of fuel samples having microorganism

The number of microorganism (CFU/ml)	The ratio of fuel sample with microorganisms (%)					
	Aerobic bacteria	Anaerobic bacteria	JetA1 degrading microorganism	Actinom yces	Fungi	Yeast
$> 10^4$	10,5	0	1,2	0	0.6	0
$10^2 - 10^4$	19,3	8,3	21,5	19,2	11,3	15,0
$10^1 - 10^2$	20,1	41,6	27,2	30,7	38,0	35,0
$<10^1$	50,0	50,0	49,4	50,0	50,0	50,0

Table 5. The number of microorganism in jet fuel filter

Samples	Aerobic bacteria	JetA1 degrading bacteria	JetA1 degrading fungi	Actino-myces	Fungi	Yeast
A320	3×10^8	2×10^5	10^4	10^4	10^7	10^7
ATR-72	10^7	10^5	10^4	10^4	107	10^7

According to the table 3 and 4, the number of microorganism groups is in this order: Aerobic bacteria > fungi > JetA1 utilizing bacteria > actinomyces > yeast > anaerobic bacteria.

All of these microorganisms can take part in the JetA1 fuel metabolism. Some strains are able to degrade fuel hydrocarbons; others have capacity of utilizing mediator products produced by directly fuel utilizing bacteria. Therefore, the presence of all microorganisms affect on quality of fuel, especially on ash weight after burning fuel. This amount of ashes is mechanics mingling which makes the engine corrosive and shorten the life.

We analyzed samples from all airplanes of Vietnam airlines. According to the data in table 6, we rely that the plane A320 and TU134 are the most highly injected microorganisms (56-57%). Particularly, it is very important to pay attention to JetA1 utilizing aerobic bacteria and fungi, because they created viscous membrane in filter, so they make filter obstructed.

Table 6. The portion of infected microorganisms in jet fuel

Type of planes	The number of analyzed samples	The number of injected samples	Portion (%)
A320	109	61	56,0
Boeing	22	5	22,7
Fokker	20	5	20,0
ATR72	30	7	23,3
TU134	7	4	57,0

B. Estimating the influence of microorganisms on chemical component of fuel

Table 7. The change of saturated hydrocarbon of JetA1 caused by microorganisms

The number of carbon	Control	*Pseudomonas* sp. ASB		*Pseudomonas* sp. ASJ		*Aureobasidium pullularia* TX_1B_1	
		Hydrocarbon remaining (mg/l)	% utilized	Hydrocarbon remaining (mg/l)	% utilized	Hydrocarbon remaining (mg/l)	% utilized
C7	0	0	-	0	-	0	-
C8	0	0	-	11	-	0	-
C9	67	5	92,54	216	-	7	89,55
C10	1304	362	72,24	695	25,54	333	74,46
C11	1939	780	59,77	1125	17,79	993	48,79
C12	2012	970	51,79	1156	9,24	1486	26,14
C13	1404	766	45,44	839	5,20	1271	09,47
C14	826	482	41,65	557	9,08	733	11,26
C15	326	137	57,98	222	26,07	283	13,19
C16	103	36	65,05	82	44,66	90	12,62
C17	46	7	84,78	33	56,52	39	15,22
C18	9	0	100,00	13	-	18	-
C19	trace	0	-	0	-	0	-
Total	8036	3545	55,89	4949	17,47	5253	34,63

According to the table 6, all 3 trains highly utilize saturated hydrocarbon of JetA1 (from 17.47% to 55.89%), especially C10 – C17 hydrocarbon chains. *Pseudomonas* sp. ASB can degrade 100% C18, *Pseudomonas* sp. ASJ degrades C15 – C17 hydrocarbon chain, and *Aureobasidium pullularia* TX_1B_1 degrades C9 – C11 hydrocarbon chain. Both bacteria and fungi change the JetA1 hydrocarbon component.
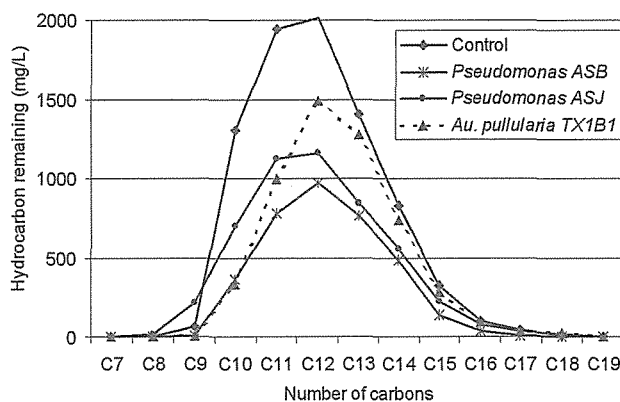

Fig.3. Degradation of saturated hydrocarbon of JetA1 by isolated microorganisms

C. Analyzing the microorganisms and composition of fuel filter samples

Table 8. Analyzing the amount of organic compound in sediment of ASG plane filter

Sample	The amount of sediment (g/ml)	Percentage
Sediment of filter before burning	0,0327	-
Sediment of filter after burning	0,0124	-
The amount of organic compound	-	62,1
The amount of inorganic compound	-	37,9

Organic component usually is from 50 to 60% in sediment of filter, if this component is higher than 60%, this means that after burning, the amount of organic compound is created by microorganisms. When the plane works this organic compound will make filter obstructed.

5. The capacity of applying

- We applied to control the number of microorganisms in jet fuel of Vietnam airlines

- We can apply to all storing units, petroleum and fuel storing in Vietnam

Figure 4. Airplane filter

Conclusion

From analyzing more than 200 jet fuel samples, we concluded:

1.   The number of microorganisms in trace water jet fuel is from 10^4 to 10^8 CFU/ml and the number of microorganisms is only from 10^1 to 10^3 in samples without water.

2.   Basing on morphology and biophysical, biochemical characteristics, microorganisms found in Jet A1 fuel are: *Aeromonas, Chryseomonas, Pseudomonas, Bacillus, Mycobacterium, Micrococcus, Candida, Rhodotorula, Aspergillus, Penicilium, Aureobasidium, Paecillomyces, Acremonium, Streptomyces*

3.   According to the result of analyzing organic and inorganic compound in filter, microorganisms are the reason that creates viscous membrane which make fuel obstructed. The main microorganism structures on fuel filter are fungi and bacteria.

4.   Analyzing hydrocarbon component of JetA1 under the effect of two bacterial and one fungus strain, the capacity of growing and changing fuel component of microorganisms brings about the decrease the fuel quality.

5.   It is necessary to control the number of microorganisms during fuel storing frequently, drain water periodically and treat reservoirs with biocide when the number of bacteria is higher than 10^2 CFU/ml.

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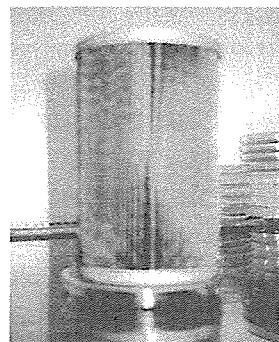Defene standard turbine fuel, aviation kerosene type JET A1. May 1996, DEF/STAN 91-91/2 (DERD 2494).

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