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Biodegradation of International Jet A-1 Aviation Fuel by Microorganisms Isolated from Aircraft Tank and Joint Hydrant Storage Systems

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Abstract Microorganisms contaminating international Jet A-1 aircraft fuel and fuel preserved in Joint Hydrant Storage Tank (JHST) were isolated, characterized and identified. The isolates were *Bacillus subtillis*, *Bacillus megaterium*, *Flavobacterium oderatum*, *Sarcina flava*, *Micrococcus varians*, *Pseudomonas aeruginosa*, *Bacillus licheniformis*, *Bacillus cereus* and *Bacillus brevis*. Others included *Candida tropicalis*, *Candida albicans*, *Saccharomyces estuari*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Cladosporium resinae*, *Penicillium citrinum* and *Penicillium frequentans*. The viable plate count of microorganisms in the Aircraft Tank ranged from 1.3 (±0.01) × 104 cfu/mL to 2.2 (±1.6) × 104 cfu/mL for bacteria and 102 cfu/mL to 1.68 (±0.32) × 103 cfu/mL for fungi. Total bacterial counts of 1.79 (±0.2) × 104 cfu/mL to 2.58 (±0.04) × 104 cfu/mL and total fungal count of 2.1 (±0.1) × 103 cfu/mL to 2.28 (±0.5) × 103 cfu/mL were obtained for JHST. Selected isolates were re-inoculated into filter sterilized aircraft fuels and biodegradation studies carried out. After 14 days incubation, *Cladosporium resinae* exhibited the highest degradation rate with a percentage weight loss of 66 followed by *Candida albicans* (60.6) while *Penicillium citrinum* was the least degrader with a weight loss of 41.6%. The ability of the isolates to utilize the fuel as their sole source of carbon and energy was examined and found to vary in growth profile between the isolates. The results imply that aviation fuel could be biodegraded by hydrocarbonoclastic microorganisms. To avert a possible deterioration of fuel quality during storage, fuel pipe clogging and failure, engine component damage, wing tank corrosion and aircraft disaster, efficient routine monitoring of aircraft fuel systems is advocated.

Keywords Aviation fuel · Biodegradation · Microorganisms · Corrosion · Aircraft disaster

Aviation fuel is a mixture of hydrocarbons whose boiling point is below 200°C. It is obtained from practical distillation of petroleum. It is known to volatilize readily into the air to form a flammable mixture (Speight 1993). The fuel is primarily a mixture of alkanes (paraffins), hexane, heptane and octane.

Since 1930, the petroleum industry had recognized the harmful effects of microbial growth in refined fuel as a world wide problem. The problem of microbial contamination and deterioration of aviation fuel is of special concern in view of the rigorous standards of fuel quality demanded by Jet aircraft. Fuel probe fouling, filter plugging, topcoat deterioration, engine component damage and wing tank corrosion have all been associated with the presence of microorganisms in aircraft fuel systems (Elphick 1975; Park 1975).

Anon (1976) reported fuel pump failures in some aircraft in the Suez in 1952 due to sulfide corrosion caused by growth of sulphate-reducing bacteria in the water bottoms of gasoline storage tank. The microbiology of petroleum hydrocarbon (Odu 1972; Antai and Mgbomo 1989; Ijah and Ukpe 1992; Atlas and Bartha 1972; Okpokwasili and Okorie 1988; Itah 1999; Itah and Essien 2001; Essien et al. 2003; Brooks and Itah 2004) and of tar balls degradation

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(Itah and Essien 2005) have been well documented. There are reports on the microbiological degradation of aircraft fuel (Perber 1971; Elphick 1975; Park 1975; Berner and Ahearn 1977). The microorganisms implicated in deterioration of aviation fuel systems include primarily members of the genera *Cladosporium* and a lesser extent the aerobic endospore bearing bacteria such as *Bacillus* species. Walker and Cohwell (1975) reported the degradation of South Lousiana fuel by *Pseudomonas*, *Corynebacteria*, *Candida* and *Penicillium* species.

There are scanty reports on the microbiology of aviation fuel in Nigeria. This paper therefore examines the microbiological quality of aviation fuel from the aircraft and storage tank in a local airport in Nigeria.

Materials and Methods

International Jet A-1 aviation fuel was aseptically collected from a local airport into sterile plastic containers. Two samples were collected at different points; one from the Aircraft Tank (AT) while the other was taken from unused fuel in the Joint Hydrant Storage Tank (JHST). The samples were analysed within 6 h of collection. This monitoring was carried out weekly for 10 weeks.

The total heterotrophic bacteria, fungi and aviation fuel degraders were determined using the pour plate technique and aliquots of decimal dilutions of the fuel from AT and JHST, respectively. The medium of choice for bacterial isolation was tryptone soya agar (TSA) supplemented with filter sterilized cycloheximide antibiotics (40 g/mL) (Stigma Chemical Co., USA) to inhibit fungal growth. Total heterotrophic mycological count was determined on Potato Dextrose Agar (PDA) supplemented with 0.5 mg streptomycin/L to inhibit bacterial contaminants while the Mineral Salt Agar (MSA) was used for total aviation fuel degrading microorganisms using the vapour phase transfer (VPT) technique (Ijah and Ukpe 1992; Itah 1999; Itah and Essien 2001; Essien et al. 2003; Brooks and Itah 2004; Itah and Essien 2005). The inoculated plates were incubated at room temperature of 26°C to 27°C for enumeration of bacteria (2 days), fungi (5 days) and fuel degraders (14 days). The above media were also heavily seeded with 0.1 mL of the aviation fuels for direct isolation of contaminants.

Representative growth colonies on each plate were repeatedly subcultured and purified on nutrient agar plates by streak plating technique. Purified isolates were characterized by a determination of the Gram's reaction, biochemical tests and carbohydrate fermentation profile. Their identities were determined using standard taxonomic schemes for bacterial (Holt et al. 1994; Barrow and Feltham 2003) and fungal (Lodder 1974; Barnet and Hunter 1987) identification.

The growth rates of selected isolates were determined using aviation fuel supplemented medium. Nearly 200 mL of sterile Mineral Salt Medium (MSM) was dispensed into

Table 2 Mean count of aviation fuel degrading microorganisms

Sample no. (weeks)	Aviation fuel	
	Aircraft tank	J. H. storage tank
1	$1.4(\pm0.6) \times 10^2$	10^3
2	10^2	10^3
3	10^2	10^3
4	$1.3(\pm0.5) \times 10^2$	$1.1(\pm0.2) \times 10^3$
5	10^2	$1.3(\pm0.4) \times 10^3$
6	10^2	$1.0(\pm0.1) \times 10^3$
7	$2.1(\pm0.4) \times 10^2$	$1.0(\pm0.4) \times 10^3$
8	$1.2(\pm0.3) \times 10^2$	$1.2(\pm0.8) \times 10^3$
9	10^2	$1.3(\pm0.2) \times 10^3$
10	10^2	$1.0(\pm0.6) \times 10^3$

Table 1 Viable plate count of microorganisms isolated from named aviation fuel

Sample no. (weeks)	Aviation fuel			
	Aircraft tank		J. H. storage tank	
	Bacteria	Fungi	Bacteria	Fungi
1	$1.3(\pm0.01) \times 10^4$	10^2	$1.79(\pm0.2) \times 10^4$	$2.1(\pm0.1) \times 10^3$
2	$1.22(\pm0.2) \times 10^4$	10^2	$2.46(\pm0.3) \times 10^4$	$1.5(\pm0.2) \times 10^3$
3	$1.48(\pm1.23) \times 10^4$	$2.01(\pm0.2) \times 10^3$	$2.66(\pm0.05) \times 10^4$	$3.3(\pm0.4) \times 10^3$
4	$1.4(\pm0.4) \times 10^4$	$1.28(\pm1.3) \times 10^3$	$3.46(\pm0.6) \times 10^5$	$2.8(\pm0.1) \times 10^3$
5	$1.48(\pm0.04) \times 10^4$	$2.38(\pm0.3) \times 10^3$	$3.58(\pm0.06) \times 10^5$	$3.46(\pm0.01) \times 10^3$
6	$1.5(\pm1.6) \times 10^4$	$1.8(\pm0.52) \times 10^3$	$4.2(\pm0.05) \times 10^5$	$1.22(\pm0.1) \times 10^3$
7	$1.4(\pm1.6) \times 10^4$	10^3	$1.65(\pm0.2) \times 10^4$	$2.3(\pm0.4) \times 10^3$
8	$1.24(\pm1.3) \times 10^4$	$1.35(\pm0.56) \times 10^3$	$2.96(\pm0.03) \times 10^5$	$3.1(\pm0.02) \times 10^3$
9	$1.28(\pm0.3) \times 10^4$	$1.89(\pm0.48) \times 10^3$	$3.8(\pm0.06) \times 10^5$	$2.48(\pm.03) \times 10^3$
10	$2.2(\pm1.6) \times 10^4$	$1.68(\pm0.32) \times 10^3$	$2.58(\pm0.04) \times 10^4$	$2.28(\pm0.5) \times 10^3$

Figures represent means of three replications in cfu/mL

± Standard error

Table 3 Frequency of occurrence of microbial isolates in named aviation fuel

Microorganisms	Aviation fuel		
	Joint hydrant storage tank (JHST)	Aircraft tank (AT)	Growth in fuel-mineral salt broth
Bacillus subtilis	8	10	+++
Bacillus megaterium	3	6	++
Flavobacterium oderatum	8	8	++
Sarcina flava	4	8	++
Micrococcus varians	6	5	+++
Pseudomonas aeruginosa	10	10	+++
Bacillus licheniformis	1	2	++
Bacillus cereus	3	6	++
Bacillus brevis	4	2	++
Candida tropicalis	8	9	+++
Candida albicans	10	8	+++
Saccharomyces estuari	4	7	++
Saccharomyces cerevisiae	6	8	++
Schizosacchanomyces pombe	8	7	+++
Aspergillus flavus	5	8	+++
Aspergillus niger	3	6	+
Aspergillus fumigatus	2	3	+++
Cladosporium resinae	8	9	+++
Penicillium citrinum	6	7	++
Penicillium frequentans	2	4	++

+++ Abundant growth

++ Moderate growth and

+ Minimal growth

Figures indicate frequencies of occurrence

sterile 250 mL capacity Erlenmeyer flasks. To each of the flasks was seeded 3 mL of filter sterilized aviation fuel and 0.2 mL of 18 h old tryptone soya broth culture of test organisms. Uninoculated flasks served as control for each culture. The cultures were incubated on orbital shaker (SGM-300, Gallenkamp, England) at 120 rev/min for 14 days. During incubation, representative samples were taken 48 hourly for determination of pH optical density (OD), temperature and the residual aviation fuel. The OD was determined at 560 nm wavelength using the Spectrophotometer (Spectronic 20. Milton Roy Co., New York) while the residual aviation fuel was determined gravimetrically using diethyl ether as the extraction solvent. This method has earlier been adopted by many authors (Odu 1972; Ijah and Ukpe 1992; Itah and Essien 2005). For each sample, 5 mL of diethyl ether was added vigorously shaken manually. The mixtures were then separated using a laboratory funnel and evaporated at room temperature to remove the solvent thus leaving the residual aviation fuel. The weights of the aviation fuel residues were then determined using a standard curve. The percentage biodegradation rate of the aviation fuel was determined from the mathematical relationship:

$$\%\,\text{Degradation} = \frac{a-b}{a} \times \frac{100}{1}$$

where a is the weight of aviation fuel (control), b, the weight of aviation fuel remaining in each case.

This was determined as earlier described (Okpokwasili and Okorie 1988; Ijah and Ukpe 1992; Itah 1999; Itah and Essien 2001; Essien et al. 2003 and Itah and Essien 2005) using aviation fuel supplemented MSM. Nearly 8 mL of sterile SMS were dispensed into each sterile tube. About 0.2 mL of the filter sterilized aviation fuel were then added followed by addition of 0.1 mL (about 4.5×104 cells mL^{-1}) of 18 h tryphone soya broth culture of each test organism. The cultures were incubated undisturbed at room temperature of 26–28°C under static condition for

Table 4 Weight losses from aviation fuel resulting from growth of named hydrocarbonclastic fungi

Incubation period (days)	Percentage (%) weight losses*				
	C. albicans	*S. estuari*	*Asp. fumigatus*	*Pen. citrinum*	*Cladospor. resinae*
2	15.8 (±0.2)	12 (±0.04)	10	8.9	17.8
4	22.4 (±0.3)	19.2 (±0.1)	15.1	16.8	28.5
6	32.8 (±0.6)	32.4 (±0.8)	21.8	22.4	38.2
8	38.2 (±1.3)	36.6	31.6	28.9	46.1
10	46.2 (±0.7)	38.8	43.8	30.2 (±0.4)	52.8
12	51.3 (±0.45)	43.6	48.9	37.5 (±0.11)	62.6
14	60.6 (±0.41)	46.2	53.2	41.6 (±0.06)	66.9

* $\text{Wt. losses}\,(\%) = \frac{\text{Wt. of fuel (control)} - \text{Wt. of fuel (degraded)}}{\text{Wt. of fuel (control)}} \times 100$

Table 5 Weight losses from aviation fuel resulting from growth of named hydrocarbonoclastic bacteria

Incubation period (days)	Percentage (%) weight losses*					
	B. subtilis	*B. megaterium*	*P. aeruginosa*	*M. varians*	*S. flava*	*Flavobacterium*
2	12	10	8	6	6	5
4	15	13	8	7	8	8
6	18	16	14	12	10	12
8	25	20	16	14	13	18
10	36	31	21	20	26	20
12	40	38	30	35	36	27
14	48	45	43	44	40	38

* Wt. losses $(\%) = \frac{\text{Wt. of fuel (control)} - \text{Wt. of fuel (degraded)}}{\text{Wt. of fuel (control)}} \times 100$

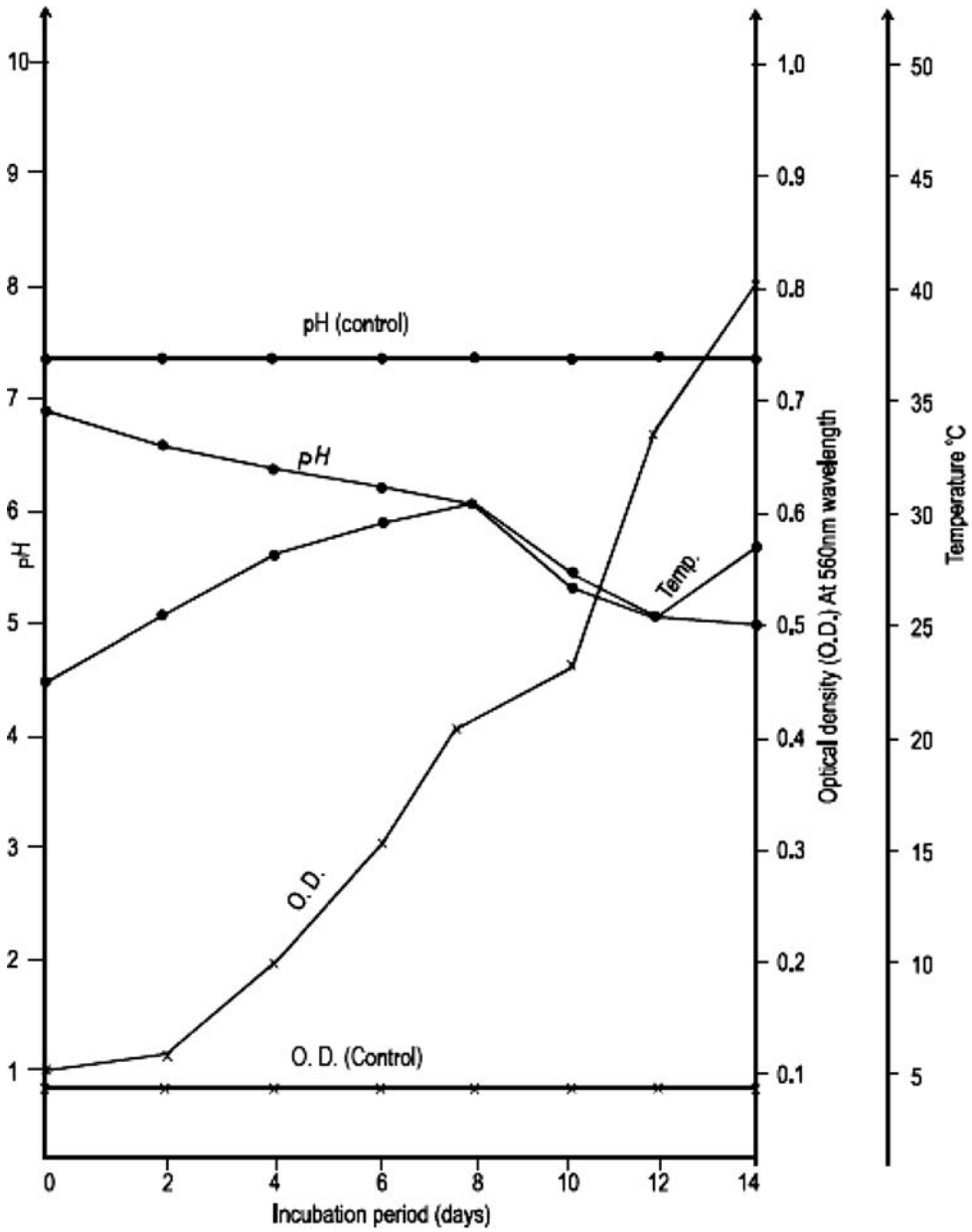

Fig. 1 Growth profile of *Bacillus subtilis* in mineral salt broth containing aviation fuel as sole source of carbon and energy

14 days. Uninoculated tubes for each test isolate served as controls. The population dynamics of the inocula in the fuel supplemented with MSM was determined 48 hourly by cultural technique as an index of the ability of the isolates to utilize aviation fuel as their sole source of carbon and energy. The growth of test isolates were monitored in media supplemented with fuels from JHST and AT, respectively. The results were scored as abundant (+++), moderate (++) and minimum (+) depending on degree of turbidity.

Results and Discussion

The mean total heterotrophic bacterial and fungal burden ranged from 1.22 (0.01) $\times$ 10^4 to 2.2 (1.6) $\times$ 10^4 cfu/mL and 10^2 to 2.38 (0.38) $\times$ 10^3 cfu/mL, respectively for AT fuel. A range of total heterotrophic bacterial count of 1.65 (0.2) $\times$ 10^4 to 4.2 (0.05) $\times$ 10^5 cfu/mL and total fungal count of 1.22 (0.1) $\times$ 10^3 to 3.3 (0.4) $\times$ 10^3 cfu/mL were obtained from JHST. It is clear from the results (Table 1) that aviation fuel whether in storage tank or the aircraft system is subject to microbial contamination and that because of their ubiquitous nature, viable microorganisms could be recovered from the fuels. However, microbial counts of 10^4 cfu/mL and above may constitute a hazard to the aircraft and innocent passengers as this could result in deterioration of fuel quality and poor engine performance.

Table 2 shows the viable plate count of aviation fuel degrading microorganisms. Mean counts ranging from

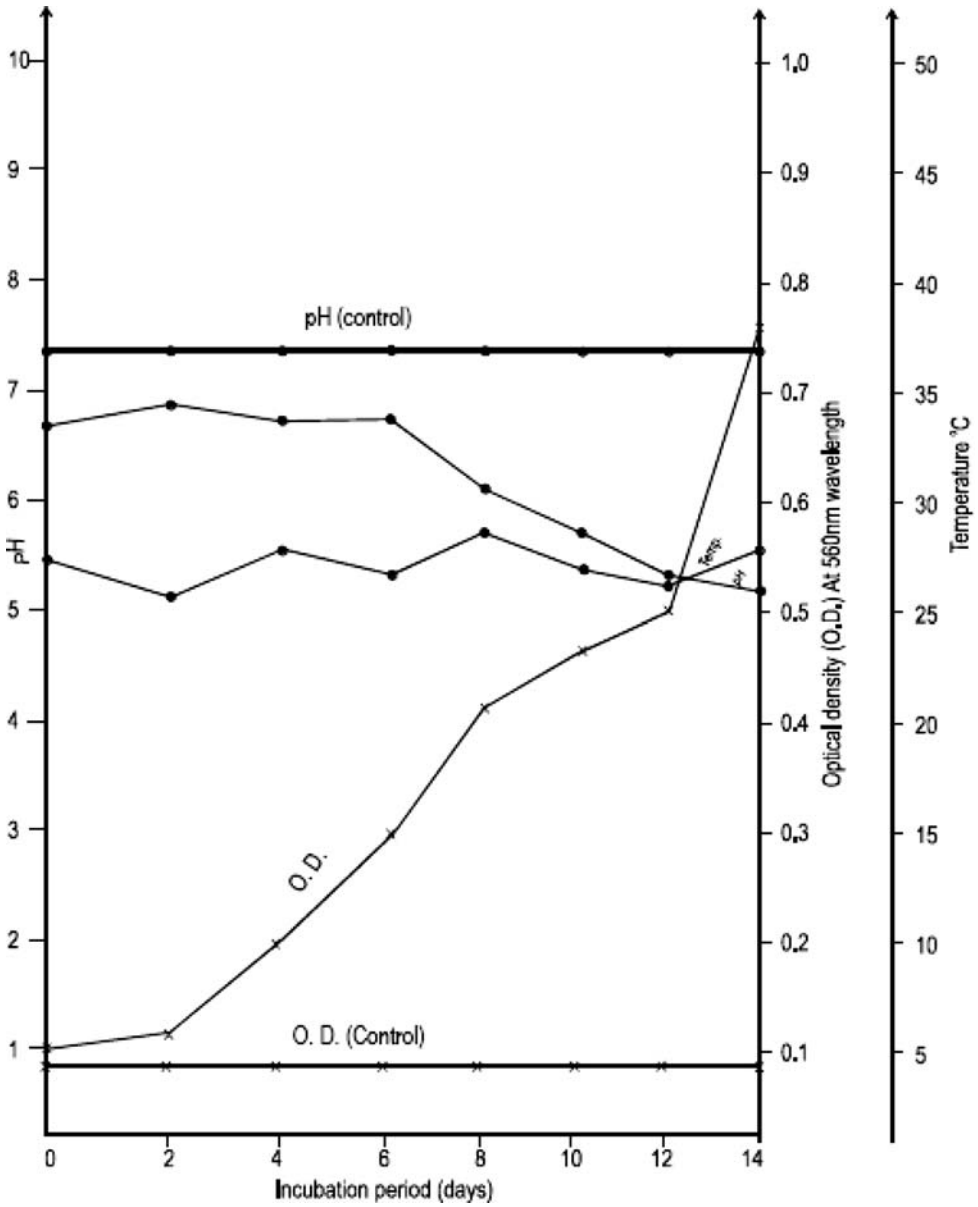

Fig. 2 Growth profile of *Bacillus megaterium* in mineral salt broth containing aviation fuel as sole source of carbon and energy

10^2 to 2.1 (0.4) $\times$ 10^2 cfu/mL and 10^3 to 1.3 (0.04) $\times$ 10^3 cfu/mL were obtained from AT and JHST, respectively over a period of 10 days. The results have revealed the presence of aviation fuel degrading microorganisms although relatively lower counts of fuel degraders were encountered in AT when compared to the JHST. The differences in counts of fuel degraders within the monitoring period of 10 days could be attributable to environmental factors like pH and temperature fluctuations and the possibility of missing out some organisms during repeated sampling protocols.

Table 3 shows the frequency of occurrence of the microbial isolates with *Pseudomonas aeruginosa* having the highest frequency of ten in both the JHST and AT systems while *Bacillus licheniformis* was the least among the bacterial isolates. The most frequently occurring fungi were *Candida albicans*, *Cladosporium resinae* and *Candida tropicalis*. The varying degrees of aviation fuel utilization and degradation by some isolates could be attributable to their ability to elaborate vital hydrogenages enzymes required for degradation of recalcitrant components of the fuel as earlier reported (Itah 1999; Itah and

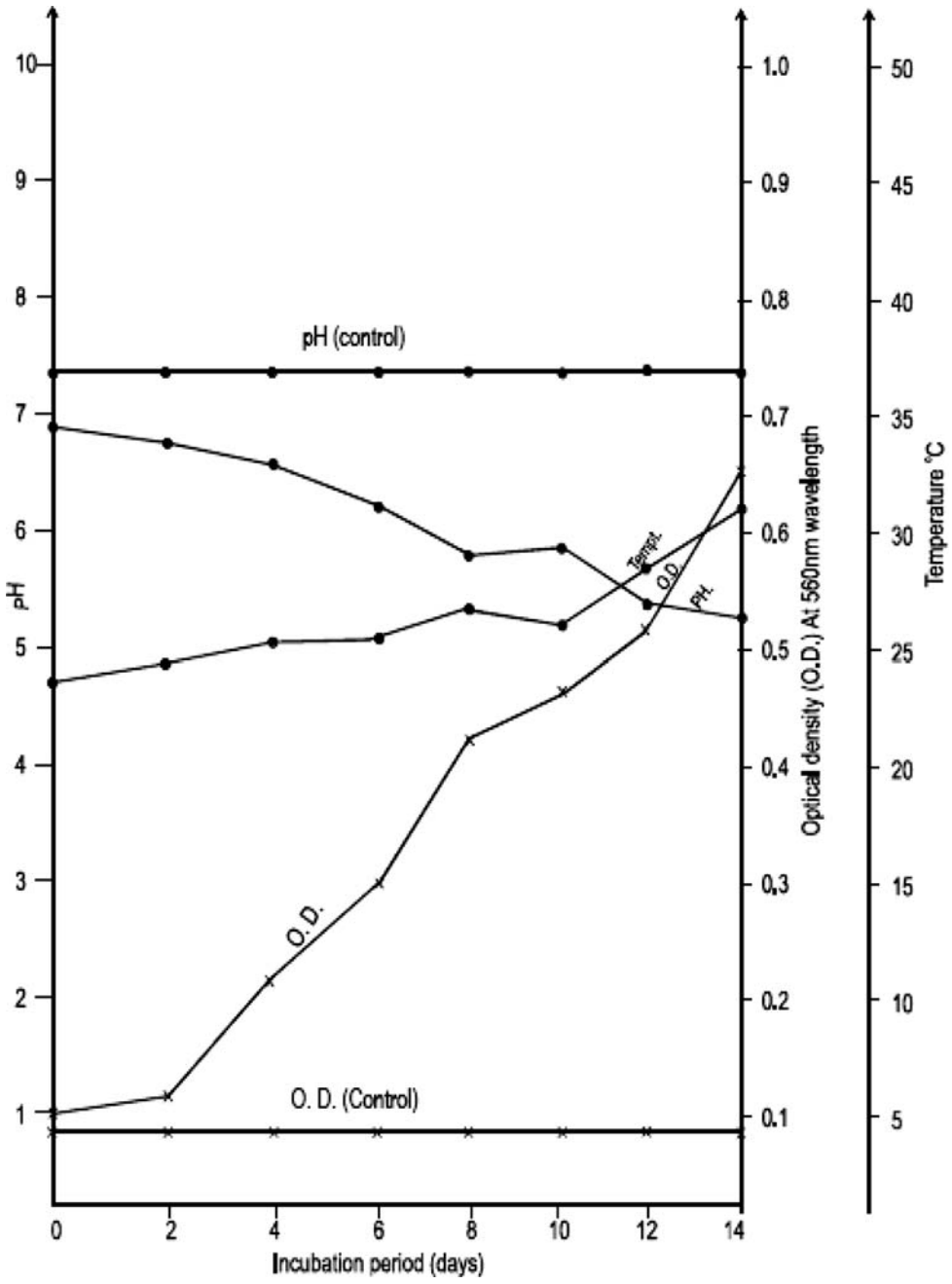

Fig. 3 Growth profile of *Pseudomonas aeruginosa* in mineral salt broth containing aviation fuel as sola source of carton and energy

Essien 2001, 2005). The isolates were *Bacillus subtilis*, *Bacillus megaterium*, *Flavobacterium oderatum*, *Sarcina flava*, *Micrococcus varians*, *Pseudomonas aeruginosa*, *Bacillus licheniformis*, *Bacillus cereus* and *Bacillus brevis*. Others were *Candida tropicalis*, *Candida albicans*, *Saccharomyces estuary*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Cladosporium resinae*, *Penicillum citrinum* and *Penicillium frequentans*. Table 4 reveals that some isolates degraded the fuels by causing weight losses of the original fuel in varying degrees and metabolized the fuel as their sole source of carbon and energy for growth and development. The fungus with the highest aviation fuel degrading potential was *Cladosporium resinae* which had a high utilization ability (+++) and caused a weight loss of 17.8%–66.9% of the original fuel over 14 days incubation period at room temperature. *Aspergillus niger* was the least with minimal growth (+). This was followed by *Candida albicans* (60.6%), *Aspergillus fumigatus* (53.2%), *Saccharomyces estuary* (46.2%) and *Penicillium citrinum* (41.6%). Comparable results were obtained for bacteria with *B. subtilis* having the highest fuel utilization (+++) and degrading capabilities ranging from 12% to 48% after 14 days incubation at room temperature. This was closely followed by *B. megaterium* (10%–45%), *P. aeruginosa* (8%–43%), *M. varians* (6%–44%), *S. flava* (6%–40%) and *Flavabacterium oderatum* (5%–38%) Table 5.

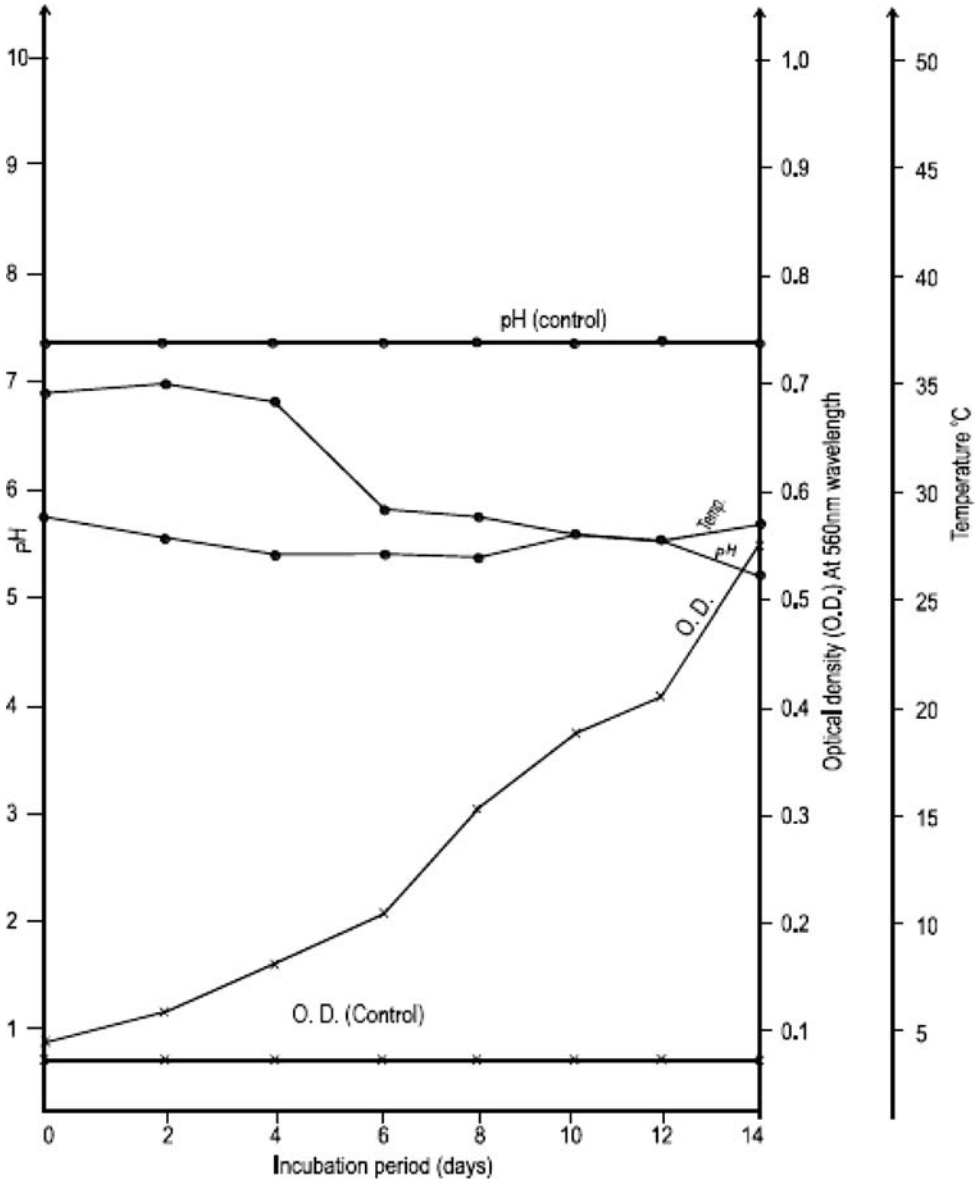

Fig. 4 Growth profile of *Micrococcus varians* in mineral salt broth containing aviation fuel as sole source of carbon and energy

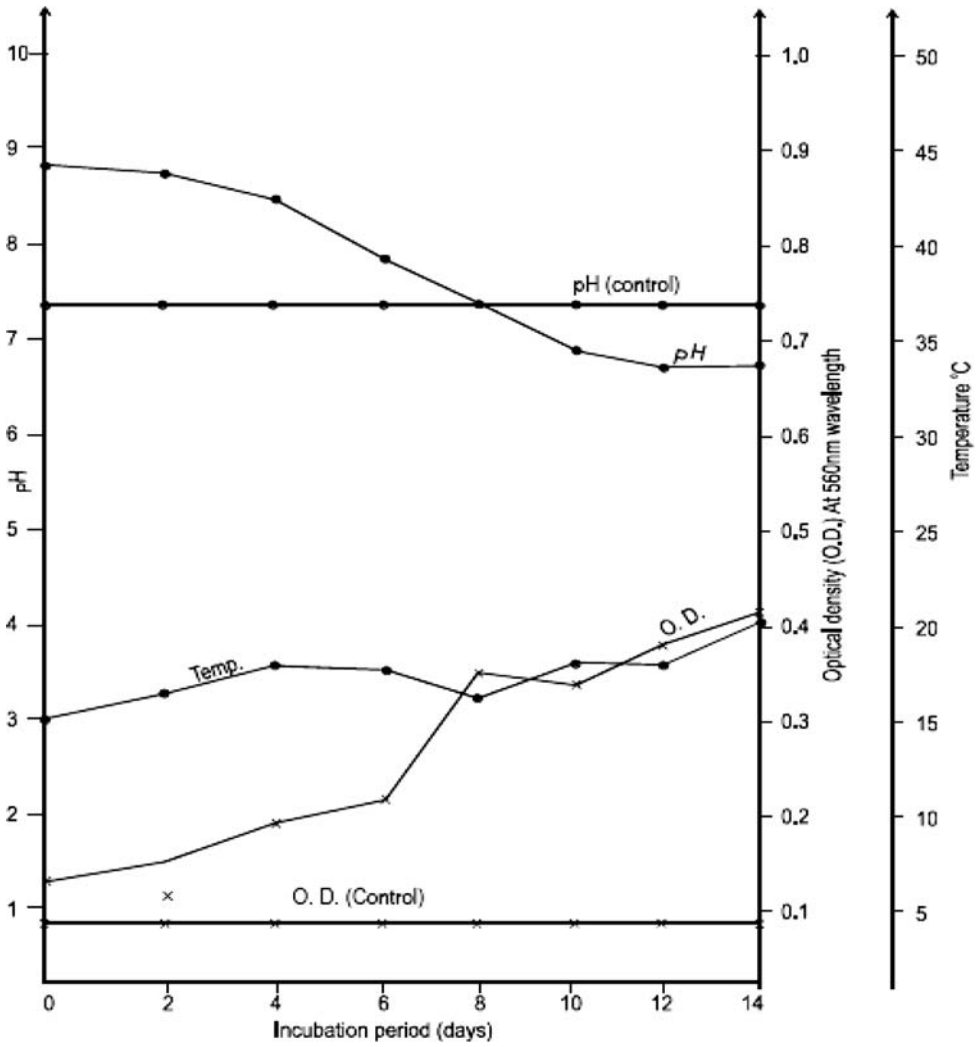

Fig. 5 Growth profile of *Sarcina flava* in mineral salt broth containing aviation fuel as sole source of carbon and energy

The growth profile of selected aviation fuel degrading bacteria based on their optical density (OD) at 560 nm wavelength, pH and temperature of cultures are presented in Figs 1, 2, 3, 4, 5 and 6 for *B. subtilis*, *B. megaterium*, *P. aeruginosa*, *M. varians*, *S. flava* and *Flavobacterium oderatum,* respectively. *B. subtilis* exhibited the highest OD of 0.82 at 560 nm wavelength followed by *B. megaterium* (0.75) while *S. flava* had the least (0.43) over the same period (14 days) at room temperature. An increase in OD is a reliable index of measurement of growth and viability of the isolates as well as their aviation fuel utilization ability in the fuel supplemented media. The results have also revealed a decrease in pH of the growth media over time due to production of acidic metabolites and an increase in temperature of the fuel supplemented media probably because biodegradation is an exothermic process. This, however, varied depending on the microorganisms involved and their ability to produce relevant enzymes.

Okpokwasili and Okorie (1988) reported that bacteria are the principal biodegraders of fuel when pH is near neutral but give way to molds and yeasts as the pH becomes acidic. However, fungi such as *Aspergillus fumigatus*, *Penicillium* spp. and *Candida* spp. which are known to grow best at acidic pH can exhibit some degree of tolerance for bacteriological media or environment of alkaline or near alkaline pH. It is therefore not surprising to find diverse genera and species of yeasts, molds and bacteria in both fuels from AT and JHST systems studied. It would imply that aviation fuels harbour both acid tolerant bacteria as well as yeast and molds capable of tolerating alkaline pH alkaline environment. As a result of the synergistic of the microbial consortia in both fuels, degradation of fuels would be enhanced. This is in consonance with earlier observations by Walker and Cohwell (1975) reported enhanced hydrocarbon fuel degradation when there is associated growth of microorganisms. Some

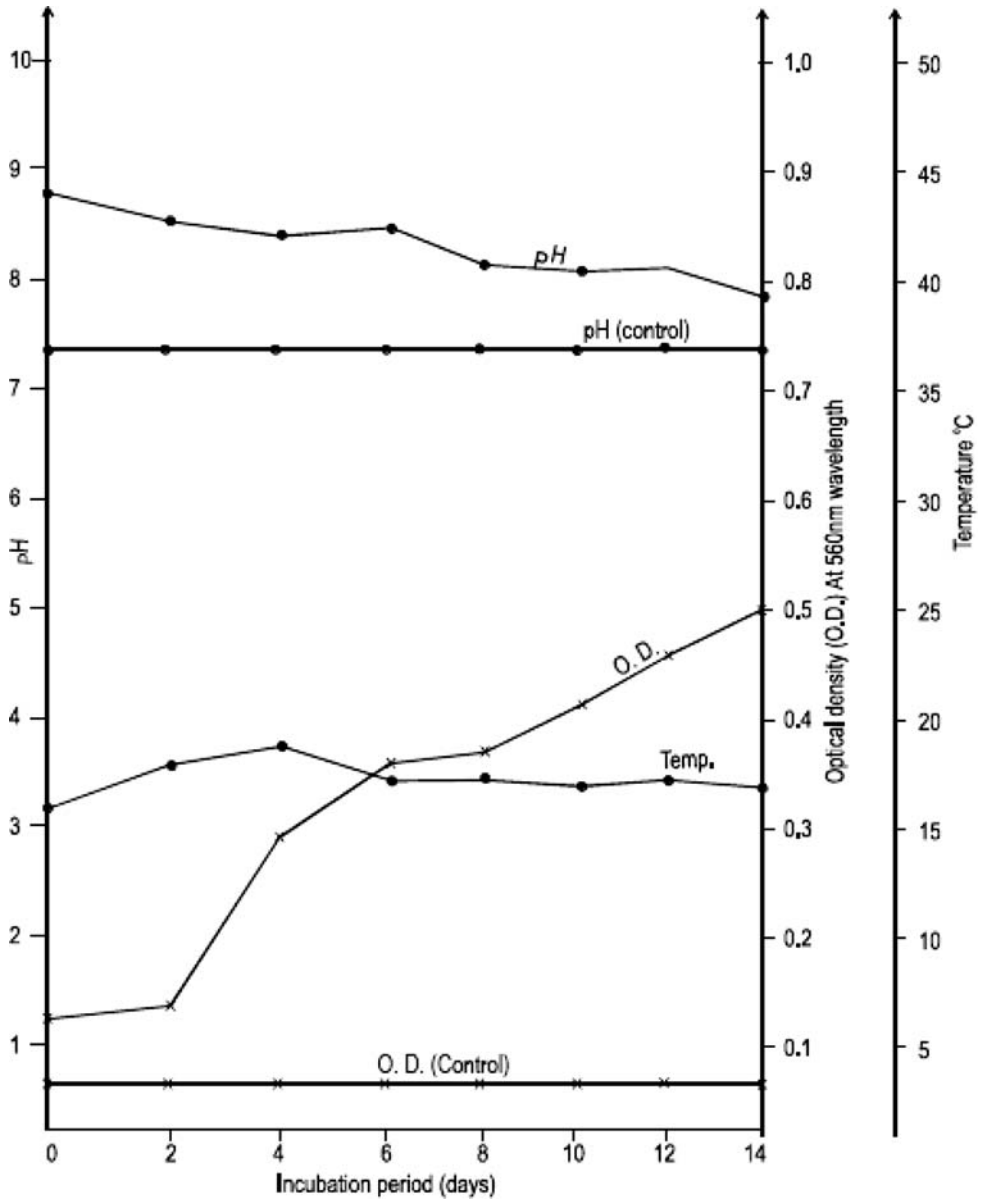

Fig. 6 Growth profile of *Flavabacterium* in mineral salt broth containing aviation fuel as sole source of carbon and energy

microorganisms possess the special attributes of initiating degradation of larger molecules to smaller fragments for subsequent degradation by others. A complete biological degradation of aviation fuel by microorganisms would yield carbon dioxide, water, sulphates, nitrates and methane as major products. Intermediate products include acids, ketones, aldehydes, alcohols, peroxides and sulphoxides which may be deposited at the bottom of the fuel tanks. Although the sulphate reducing bacterium, *Desulfovibrio desulphuricans*, which is known to cause serious problems in gasoline (aviation fuel) was not encountered in this work, the isolation of *Cladosporium resinae* and other aviation fuel degrading molds is a dangerous signal as *Cl. resinae* is associated with deterioration and degradation of 115/145 grade aviation gasoline (Goodger 1975) and could be disastrous. The mechanical strength produced by fungal mycelia is sufficient to cause filter blockage and malfunctioning of fuel gauges. Other hazardous consequences are the retention of waste and intensification of corrosive attack in water-porous AT linings.

This work has revealed that aviation fuels are not sterile after all as viable aviation fuel degrading bacteria, yeasts and molds were encountered in both the AT and JHST systems. The results suggest the need for sufficient treatment of the fuels and continuous monitoring of both fuel systems as contamination of the fuels over time could result in mysterious aircraft disasters as witnessed in Nigeria recently. Aviation fuel degraders may be regarded as silent killers.

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