Desalination and Water Treatment
www.deswater.com
doi: 10.1080/19443994.2015.1046943

57 (2016) 12108–12117
June

Taylor & Francis
Taylor & Francis Group

Isolation and preliminary identification of actinomycetes isolated from a wastewater treatment plant and capable of growing on methyl ethyl ketone as a sole source of carbon and energy

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Received 3 June 2014; Accepted 26 April 2015

ABSTRACT

Volatile organic compounds are considered as major sources of air pollution. They cause toxicity problems, bad odors, global warming, etc. Methyl ethyl ketone (MEK) is used in the formulation of lacquer type paints, varnishes, cleaners, thinners, etc. and in many other industries such as the manufacture of synthetic leather and in the decaffeination of coffee. Released into the environment, it causes respiratory, eye, and skin health problems. At high concentrations, it poses a potential threat to public health. In recent years, effective, very environmentally sound, and economical organic biological waste gas treatment processes have emerged. The sources of degrading micro-organisms are diverse and activated sludge suspensions are widely used. Actinomycetes are known for their ability to degrade various polymers. In this study, we are interested in isolating *actinobacteria* from activated sludge from the wastewater treatment plant of El Athmania, Mila. Thus, five actinomycetes were isolated on ISP4 medium supplemented with nystatin at 100 μg/ml and nalidixic acid at 10 μg/ml. These isolates proved to degrade efficiently MEK in batch reactors. Growth kinetics were determined for each isolate. The time course of MEK consumption was also measured by gas chromatography. A strain named A5.7 stood out as the best degrading bacterium. Indeed, complete degradation of the substrate was achieved after only 72 h of incubation. The A5.7 isolate was assigned by morphological and cultural methods to the genus *Streptomyces*.

Keywords: Methy ethyl ketone; Biodegradation; Activated sludge; Actinomycetes; *Streptomyces*

1. Introduction

Methyl ethyl ketone (MEK) is a largely used solvent that serves as a constituent of paints, varnishes, inks, glues, and adhesives. It is released into the environment from industrial and domestic uses threatening, therefore, air quality and public health. MEK appears to be mobile, bioaccumulative and it can easily leach in to soil and cause alteration of ground water quality. Its impact on human health

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may range from irritation of mucous membranes (ocular, nasal or pharyngeal) to digestive disorders or headaches, even central nervous system dysfunction at high concentrations [1]. MEK toxicity is relatively high in the presence of other organic solvents [2]. It is thus necessary to remove MEK from the environment in order to avoid such effects.

The treatment of VOCs contaminated air has been the subject of much research and experimentation over the last decades in the hope of finding the most effective and less expensive solutions. In addition to the diverse physical and chemical treatment technologies in use, the last years have seen the emergence of very efficient biological processes that are environmentally and economically sound.

Activated sludge comprises both organic and inorganic components. It is considered as an important reservoir of micro-organisms. Actinomycetes are present in this ecosystem [3–8]. Such bacteria are widespread in nature and known to play a significant role in the biodegradation of organic and inorganic matter. Research works highlighting the responsibility of micro-organisms in the biodegradation of MEK are relatively scarce and fewer studies have concerned actinomycetes.

The aim of this study is to isolate and identify actinomycetes from activated sludge and determine their ability to grow on MEK as the sole source of carbon and energy. It falls within a research program which aims to highlight the role played by actinomycetes isolated from wastewater treatment plants in the biodegradation of certain volatile organic compounds. We present in this work preliminary results of MEK biodegradation [8].

2. Materials and methods

2.1. Isolation and enumeration of actinomycetes

Bottles with a capacity of 250 ml were used to store samples of activated sludge collected from the wastewater treatment plant of El-Athmania in the vicinity of the town of Mila in the east of Algeria. Samples were stored in the refrigerator at 4°C.

The ISP4 isolation medium was used. It consisted of soluble starch, 10 g; K_2HPO_4, 1 g; $MgSO_4$ $7H_2O$, 1 g; NaCl, 1 g; $(NH_4)_2$ SO_4, 2 g; $CaCO_3$, 2 g; the trace element solution, 1 ml; Agar, 20 g; pH, 7 to 7.4. Sterilization of this medium was carried out at 120°C during 20 min. The saline solution consisted of: $FeSO_4$ $7H_2O$, 0.1 g; $MnCl_2$ $4H_2O$, 0.1 g; $ZnSO_4$ $7H_2O$, 0.1 g; distilled water, 100 ml. Nystatin at 100 μg/ml and nalidixic acid at 10 μg/ml were added to the medium. The antibiotics used were sterilized by filtration through a millipore filter of 0.22 microns porosity. The inoculation was carried out using a sterile Pasteur pipette; 0.1 ml of each decimal dilution (until 10^{-6}) was collected and inoculated on the surface of a Petri dish containing an ISP4 medium. The dishes were incubated at a temperature of 28°C for three weeks. After 21 d of incubation, bacterial colonies were counted using a colony counter. Actinomycetes were identified by their characteristic macroscopic appearance (hard colonies embedded in agar) and by direct observation of colonies under an optical microscope (Leica DMLS) at magnification ×10 (presence of thin filaments) [9]. These isolates were then observed using the same microscope at a magnification of ×100 after Gram staining. The actinomycetes were Gram+ filamentous forms sometimes fragmented into rods or cocci. Purified isolates of actinomycetes were preserved by freezing at −20°C in the presence of 20% glycerol.

2.2. Screening isolates of actinomycetes growing on MEK

A first test was performed on all isolates of actinomycetes to test their capacity to degrade the MEK as the sole carbon source. The bioreactor used in this study was in the form of a glass bottle with a total volume of 500 ml, closed with a perforated rubber stopper which allowed insertion of a syringe needle to collect samples. The minimal medium used was that of Vandermesse [10]. This medium was completely devoid of carbon source. Its chemical composition was as follows: KNO_3, 13.76 g/l; KH_2PO_4, 1.78 g/l; Na_2HPO_4 $2H_2O$, 4.66 g/l; Na_2SO_4, 9.68 g/l; $MgSO_4$ $7H_2O$, 0.8 g/l; EDTA, 10 mg/l; $FeSO_4$ $7H_2O$, 5 mg/l; $MnCl_2$ $4H_2O$, 1.22 mg/l; $ZnSO_4$ $7H_2O$, 0.25 mg/l; $CuSO_4$ $5H_2O$, 0.2 mg/l; $CaCl_2$ $2H_2O$, 1 mg/l; Na_2MoO_4 H_2O, 0.2 mg/l.

The inoculum was prepared in the same manner for all bacteria. From bacterial cultures, two platinum loops were removed aseptically and transferred into a test tube containing 15 ml of sterile distilled water. The tubes were then agitated with a vortex for homogenization and stored at 4°C for a period not exceeding 3 h. Strains in this state served as an inoculum for the next subculture. The bacterial inoculum (15 ml), 75 ml of culture medium and 100 μl of MEK were introduced into the 500 ml sterile reactors [7,11,12]. The latter was placed in an agitated water bath and set at 30°C under stirring at 150 rev/min. Samples of 2 ml were collected every 6 h using a sterile syringe. These samples were used to measure optical density (OD). The kinetics of growth (OD = f [t]) of each isolate was then drawn using Microsoft Windows OriginLab software.

2.3. Batch culture

The preparation of the starter culture was carried out as previously described. The incubation within the reactor was carried out in an agitated water bath that was adjusted to 30°C. Samples were collected every 6 h to monitor the kinetics of growth of each isolate. A volume of 15 ml of subculture giving the first cells in their exponential phase was removed and centrifuged at 400 *g* for 20 min. The supernatant was removed and the cell biomass was added to sterile distilled water to 15 ml. This amount was used to inoculate the aerobic batch-operated reactors containing the above-mentioned volumes of medium and MEK. After incubation in the same growth conditions, samples of 2 ml were taken every 6 h and allowed two types of measurements:

(1) −1.5 ml was used to monitor the bacterial growth by reading the OD at 546 nm. The conversion of results in g/l was achieved by comparing the optical densities with a previously drawn calibration curve.
(2) −0.5 ml was collected in a sealed tube and then centrifuged at 400 g for 20 min to obtain a supernatant free from microbial cells. The sample was then stored at 4°C in eppendorfs and used to measure the concentration of the substrate (MEK) by gas chromatography. Control reactors were tested for each isolate. They received the nutritive medium described above, but no MEK.

The time course of the MEK concentration in the liquid phase was determined by injecting 1 µl in a Schimadzu gas chromatograph, Model GC-17A, equipped with a DB-5 capillary column (30 m long, 0.25 mm internal diameter). Nitrogen gas was used as carrier gas at a flow rate of 45 ml/min and a split ratio of five. The temperatures of the injector and the flame ionization detector were 250 and 200°C, respectively. The analysis was carried out isothermally at 130°C. A standard curve was established with an external standard of 1,000 ppm of MEK. Hourly injections of 1 µl allowed establishing biodegradation parameters of MEK consumption.

2.4. Specific growth rate (µ)

The specific growth rate is defined by Monod equation (1) [13]:

$$\mu = (\mathrm{d}x/\mathrm{d}t)(1/x) \quad (1)$$

where μ = specific growth rate (h^{-1}), X = biomass concentration (g/l), t = time (h). From hourly experimental measurements, we can estimate the real value of specific growth rate through Eq. (2):

$$\mu_n = (1/X_n)(X_{n+1} - X_n)/(t_{n+1} - t_n) \quad (2)$$

2.5. Maximum growth rate μ_{max}

In order to determine the maximum growth rate $\mu_{\max}$, Eq. (3) was applied:

$$X = \mathrm{P1}/\left[1 + \exp(-\mathrm{P2} + \mathrm{P3} \cdot t)\right] + \mathrm{P4} \quad (3)$$

where X = biomass concentration (g/l), t = time (h), P1, P2, P3, P4: parameters

The parameter sets obtained for each batch reactor, using Microsoft Windows OriginLab software, are shown in Table 1.

Each of these parameters is defined as follows:

P1: absolute difference between the concentrations (g_{DW}/l); P1 is equal to the difference between the initial and final biomass concentrations (A1 − A2).

P2: spreading of the curve along the *x*-axis (unitless). It is equal to $X_0/\mathrm{d}x$.

P3: slope of the sigmoid (h^{-1}); P3 is equal to $1/\mathrm{d}x$.

P4: final concentration of biomass (g_{DW}/l); P4 is represented by A2.

2.6. Specific rate of MEK consumption ($r_{x.MEK}$)

The specific rate of consumption of the MEK ($r_{x.MEK}$) is defined as the mass of MEK degraded per unit dry weight of biomass per unit of time (h). This degradation rate was calculated by Eq. (4):

$$(r_{x.\mathrm{MEK}\,n}) = -(1/X_n).[S_{n+1} - S_n/t_{n+1} - t_n)] \quad (4)$$

Table 1
Parameter values for each isolate obtained after parametric adjustment of the experimental curves of growth

Strains	P1	P2	P3	P4
A2.3	3.003	2.956	0.037	2.957
A3.2	3.589	4.269	0.042	3.738
A3.3	2.598	3.219	0.038	2.669
A3.9	3.534	5.382	0.090	3.755
A5.7	1.501	5.577	0.132	1.666

Note: P1: Absolute difference between the concentrations (g_{DW}/l), P2: spreading of the curve along the *x*-axis (unitless), P3: slope of the sigmoid (h^{-1}), P4: final concentration of biomass (g_{DW}/l).

where n = order number of the MEK concentration measurement, $(r_{x.MEK})_n$ = specific rate of MEK consumption at time t_n ($g_{MEK}/g_{DW}h$), X_n = biomass concentration at time t_n (g_{DW}/l), S_n = residual MEK concentration in the liquid phase at time t_n (ppm), S_{n+1} = residual MEK concentration in liquid phase at time t_{n+1} (ppm), t_n = time at the nth measurement of residual MEK concentration (h), and t_{n+1} = time at the (n+1) th measurement of residual MEK concentration (h).

2.7. Identification of the actinomycetes at the genus level

Several actinomycetes are identifiable only at the genus level through the study of the macroscopic appearance of colonies and morphological characters of the aerial mycelium and the substrate [14–17].

The macroscopic appearance (color, shape, etc.) of the colonies was observed after 21 d of incubation at 30˚C on the same medium. The color of the substrate mycelium was determined as follows: a piece of agar was cut from mature crops and then deposited on a disinfected substrate. Excess agar was removed by a razor blade and the color was noted [18].

The observation of the morphology of chains of spores, substrate mycelium, and aerial mycelium was performed according to the technique of culture on lamella. This technique consisted in carefully inserting sterile strips in an agar medium ISP2, ISP3, ISP4, or ISP5, such that they formed an angle of 45˚C with the surface of the latter. The bacterium was then inoculated against the blade in contact with the medium. After 14 d of incubation at 30˚C, the plate was removed carefully from the agar, carrying with it fragments of substrate and aerial mycelium; it was then deposited on a slide and examined under an optical microscope (G × 100) [18].

3. Results and discussion

3.1. Isolation

From the selective medium used (ISP4 containing antibiotics), colonies of powdery, dry, and hard appearance which are embedded in agar were collected. Observed under an optical microscope, these isolates showed a filamentous aspect with a Gram positive staining characteristic of actinomycetes. After subculturing, seven strains of actinomycetes were selected. These isolates were referred to by a code name as follows: A2.3, A3.2, A3.3, A3.9, A5.2, A5.3, and A5.7. The ISP4 medium used in this study was effective for the isolation of actinomycetes in this ecosystem. The starch and ammonium sulfate present in this medium, known as being favorable for the isolation of actinomycetes from soils [9,19–21], are also favorable for the isolation of these bacteria in this type of aquatic ecosystem.

The addition of the mixture of nystatin (antifungal) at 100 g/ml and of nalidixic acid (anti-Gram-) at 10 mg/ml to the selective medium has eliminated almost all of the undesirable fungal and bacterial contaminants. Nystatin is a very effective antibiotic for inhibiting the growth of fungi. Williams and Davies [15] tested this antifungal substance on fungi previously isolated from soil. They found that it inhibited the growth of most fungi at a concentration of 50 μg/ml. However, when tested with actinomycetes, this antibiotic had no inhibitory action toward their growth although its concentration was increased to 100 μg/ml.

Nalidixic acid was used in the work of Takizawa et al. [22] for the isolation of actinomycetes in marine environments. Suzuki et al. [23] found that actinomycetes can withstand nalidixic acid up to a concentration of 10 μg/ml; beyond the latter, growth inhibition can occur. According to our results, these two antibiotics are also indicated in the isolation of actinomycetes from activated sludge.

3.2. Growth kinetics of actinomycetes using MEK as the sole source of carbon and energy

Control reactors showed, for all the tested isolates of actinomycetes, that bacterial growth was insignificant. Fig. 1 shows the time course of the OD for the seven strains of actinomycetes isolated from activated sludge.

From these graphs (Fig. 1), isolates A2.3, A3.2, A3.3, A3.9, and A5.7 were the most efficient in terms of substrate consumption when MEK was the sole source of carbon and energy. A5.2 and A5.3 bacteria were not capable to degrade the same substrate in these culture conditions. The profile of the growth kinetics of the selected strains showed a generally slow incubation rate. Indeed, the process of adapting all isolates to growth conditions lasted from 12 to 36 h of incubation. The log phase occurred immediately after a lag phase which lasted between 100 and 150 h.

3.3. Determination of some parameters of the growth of selected isolates of actinomycetes

3.3.1. Evolution of the concentration of the biomass as a function of incubation time

Fig. 2 shows a remarkable reduction of the lag phase in reactor five. These results differ significantly from those found in the preliminary study. Indeed,

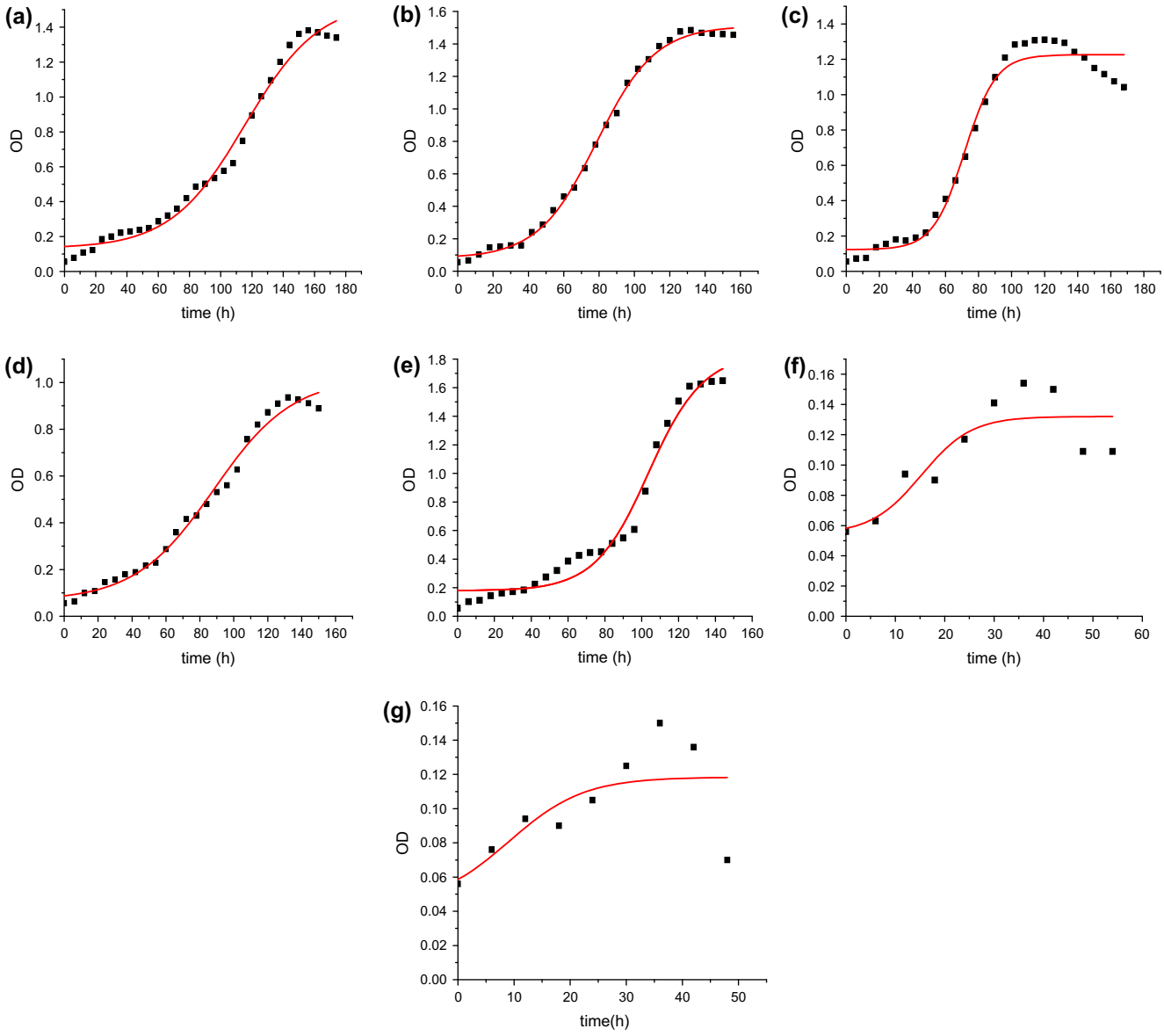

Fig. 1. Growth kinetics of different strains of actinomycetes. (a) A2.3, (b) A3.2, (c) A3.3, (d) A3.9, (e) A5.7, (f) A5.2, and (g) A5.3.

the duration of this phase was between 12 and 36 h for preliminary tests and a few hours to 18 h for the incubation period when the culture starter was used.

3.4. Determination of the specific growth rate

The time course of the specific growth rate for the different strains is shown in Fig. 3.

3.5. Determination of maximum growth rate μ_{max}

The parameter sets obtained for each batch reactor are shown in Table 1.

The quickest growing strain was A5.7. Growth lasted 72 h (3 d) (Fig. 2). The highest maximum growth rate μ_{max} was calculated for this strain (0.132 h^{-1}) (Table 1). The A5.7 strain entered the exponential phase after an acclimation period of 12 h. Once the division started, the growth of this bacterium was rapid and steady up to 42 h and then it began to slow down until stabilization occurred in the third day. The time course of the specific growth rate μ confirms these results (Fig. 2). This rate reached a maximum of 0.23 h^{-1}. A progressive reduction of growth rate down to a value of 0.16 h^{-1} was observed (Fig. 3). The maximum biomass concentration was 1.67 g_{DW}/l; it was recorded after 66 h of incubation (Fig. 2).

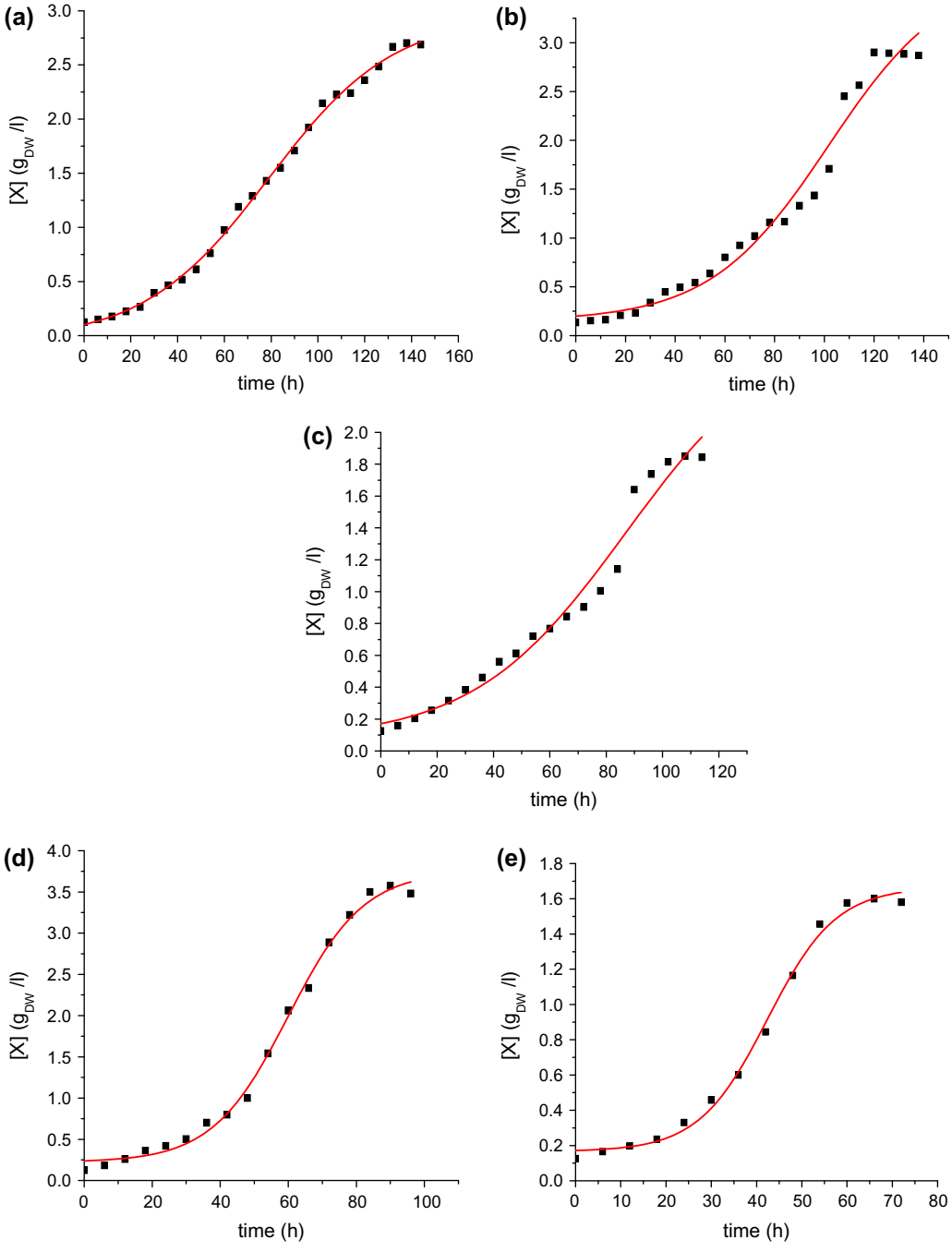

Fig. 2. Time course of the biomass concentration X (g_{DW}/l) for strains. (a) A2.3, (b) A3.2, (c) A3.3 (d) A3.9, and (e) A5.7.

3.6. Specific consumption rate of MEK ($r_{x,MEK}$) for the A5.7 strain

The results of the time course of the biomass concentration X and the residual MEK concentration for the A5.7 strain are presented graphically in Fig. 4. The profiles of the time course of the experimental specific consumption rate of MEK ($r_{x,MEK}$) are shown in Fig. 5.

3.7. The specific growth rate vs. the MEK concentration

The profiles of the specific growth rates vs. the residual MEK concentration are shown in Fig. 6. The growth of A5.7 strain varied regularly. Its specific growth rate quickly reached a maximum and then diminished gradually with the concomitant substrate concentration decrease (Fig. 4). The results show that

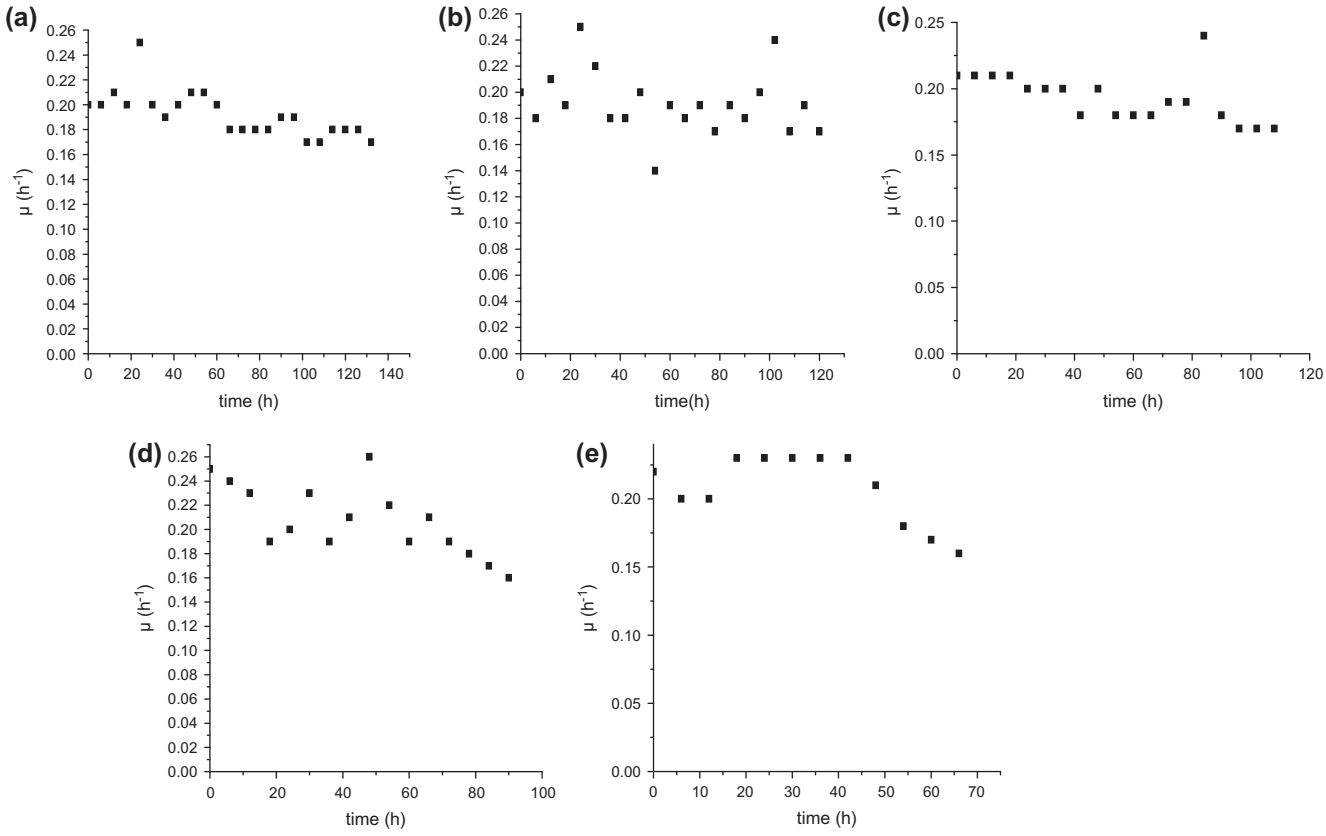

Fig. 3. Time course of the experimental specific growth rate μ (h^{-1}) for different strains. (a) A2.3, (b) A3.2, (c) A3.3, (d) A3.9, and (e) A5.7.

there is a decrease in the MEK consumption rate over time (Fig. 5).

Strain A5.7 seemed to be efficient in terms of MEK utilization. Indeed, the complete substrate degradation occurred after only 72 h of incubation (Fig. 4). Growth evolved smoothly with MEK consumption and was characterized by the highest maximum growth rate of 0.132 h^{-1} in comparison with the rest of the isolates (Table 1). The strain A5.7 was therefore selected among the set of the isolated actinobacteria.

Studies dealing with the biodegradation of MEK by a pure culture in batch reactors are very scarce [24]. Mixed cultures and consortia are commonly used [7,12,25,26]. Moreover, optimal biodegradation of volatile organic compounds is not necessarily better with a consortium. Indeed, micro-organisms isolated from activated sludge achieved faster complete methanol biodegradation than the entire sludge as an inoculum [7]. In addition, in the presence of activated sludge, complete degradation of the MEK occurred after 8 d at a concentration of 200 mg/l (200 ppm) and after 9 d at a concentration of 400 ppm [23]. At an MEK concentration of 20 mg/l in river water containing

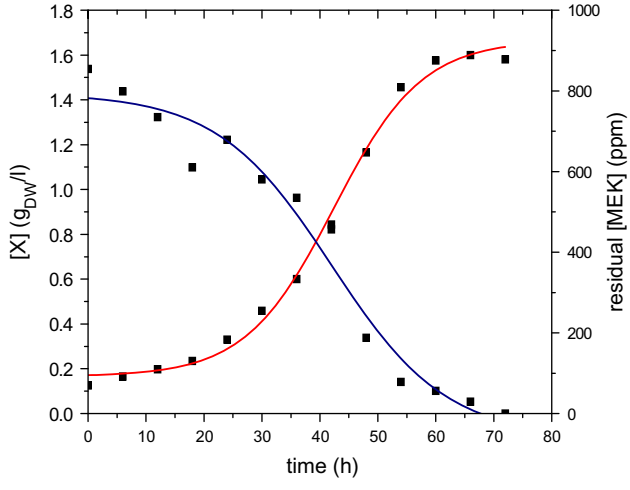

Fig. 4. The time course of the biomass concentration X (g_{DW}/l) and the MEK residual concentration (ppm) for the A5.7 strain.

micro-organisms acclimatized to MEK, complete consumption was achieved after 2.5 d [27]. Delfino and Miles [26] reported a slower rate of aerobic

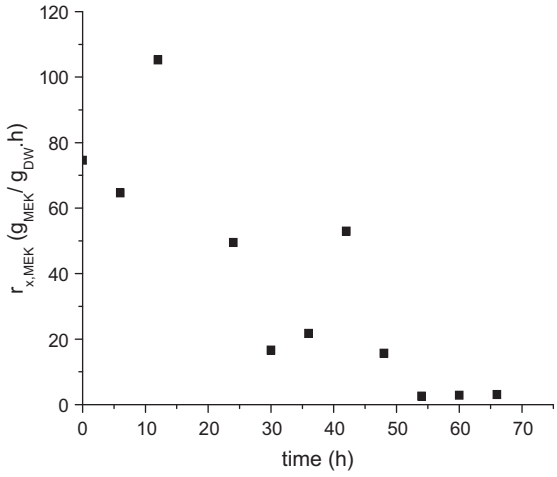

Fig. 5. The time course of the experimental specific MEK consumption rate ($r_{x,MEK}$) (g_{MEK}/g_{DW} h) for the A5.7 strain.

decomposition in groundwater; 1 mg/l was completely degraded after 14 d. Our results were quite satisfactory. Indeed, actinomycetes A5.7, which were isolated from activated sludge at El-Athmania station degraded alone the entire concentration of 900 ppm in just 72 h of incubation.

3.8. Morphological identification of strain A5.7

3.8.1. Macroscopic appearance

Owing to its speed of growth, A5.7 strain proved to be the fastest degrading bacterium compared to the other four selected isolates. The first signs of growth consisted in the appearance of round, pasty colonies which were slightly sunken in the agar after 2–3 d of incubation. After 7 d of incubation, this strain had a powdery white appearance which constituted the aerial mycelium that is characteristic of actinomycetes. From the 14th day, the aerial mycelium was gradually taking a gray color. This corresponded to the formation of mature spores at the end of aerial hyphae.

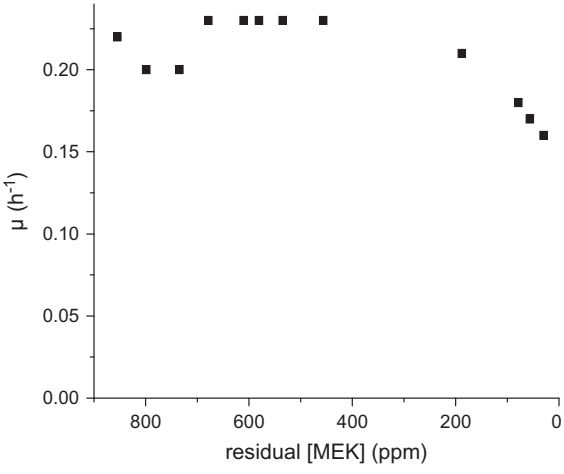

Fig. 6. The experimental specific growth rate μ (h^{-1}) vs. the residual MEK concentration for the A5.7 strain.

3.8.2. Microscopic appearance

The results of the microscopic observation of the selected strain by the inclined blades method are shown in Fig. 7. The strain A5.7 was composed of a well-developed substrate mycelium and a less dense and thicker aerial mycelium bearing long chains of spores (generally 20 spores per chain). The spore chains of cylindrical shape were either straight or spiral.

The taxonomy of actinomycetes is based on morphological, chemical, physiological, and molecular criteria. Identifying genres is facilitated by morphological studies while chemical, physiological, and molecular criteria separate species [17]. According to Williams et al. [28], certain types of actinomycetes (*Streptomyces Streptoverticillium Micromonospora, Microbispora* …) can be identified with a greater degree of accuracy compared to other genera (*Nocardia, Actinomadura* …) simply by microscopic observation. Several authors have determined the types of actinomycetes from morphological characteristics [14] simply by microscopic observation of actinomycetales strains grown on the Czapek medium. Others have identified Actinomycetales genera by observation of spore chains under an optical microscope [29].

The A5.7 strain develops colonies after 2–3 d of incubation, which is a characteristic of fast-growing actinomycetes. Nodwel and Losick [30] found that colonies of *Streptomyces coelicolor* established aerial hyphae in 24 h. The genus *Streptomyces* is part of fast-growing actinomycetes.

The A5.7 isolate constituted round-shaped colonies with a powdery appearance, which were slightly sunken in the agar. The shape of the spore chains was spiral or straight. These characteristics are typical of *Streptomyces* according to Myadoh et al. [31].

Schematized morphological aspects that characterize the genera belonging to the actinomycetes as proposed by Lechevalier [16] and which appeared in the ninth edition of Bergey's Manual, identify most of these bacteria. The appearance of aerial mycelia and A5.7 isolate (Fig. 7) is exactly the same as the genus

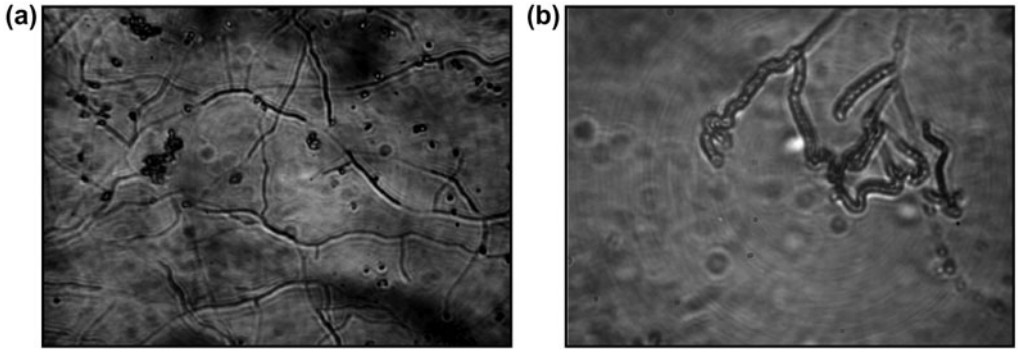

Fig. 7. Microscopic observation of A5.7 isolate under an optical microscope (Leica DMLS) (G × 100). (a) Substrate mycelium and (b) aerial mycelium with chains of spores.

Streptomyces. Therefore, on the basis of all these similarities, the isolate in question is assigned to the genus *Streptomyces*.

The role of actinomycetes in the biological treatment of volatile organic compounds is particularly known in biofilters [32–34].

4. Conclusion

Waste management is one of the most complex problems for both developed and developing countries. In addition, globalization puts new challenges for waste management within societies. Waste management is a key element for sustainable development, economic issues, health concerns, and environmental problems.

Biological treatment of VOCs containing gases is at present the most widely used technique [24,35]. Its use and development grow exponentially on an industrial scale.

In this study, several actinomycetes were isolated from activated sludge collected from the wastewater treatment plant of El Athmania, Mila. These bacteria were capable of degrading MEK. A strain assigned to the genus *Streptomyces* was the most efficient degrading bacterium of all identified isolated actinomycetes. The entire substrate degradation was achieved after 72 h of batch incubation. The calculated growth rate μ_{max} and some culture parameters reached maximum values for this strain.

Acknowledgment

We would like to express our deepest gratitude to Professor Abderrahmane Boulahrouf of the Laboratoire de Génie Microbiologie et Applications, Université Constantine 1, for allowing us to use his laboratory facilities in order to perform some of this work.

References

[1] U.S. EPA., Updated Health Effects Assessment for Methyl Ethyl Ketone, U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Cincinnati, OH, 1989.
[2] D.C. Murphy, Acute illness among workers connected to solvent exposure, Occup. Health Saf. 53(5) (1984) 36–38.
[3] M.P. Lechevalier, H.A. Lechevalier, *Nocardia amarae* sp. nov. an actinomycete common in fooming activated sludge, Int. J. Syst. Bacteriol. 24 (1994) 278–288.
[4] P. Kämpfer, Detection and cultivation of filamentous bacteria from activated sludge, FEMS Microbiol. Ecol. 23 (1997) 169–181.
[5] M. Schuppler, M. Wagner, G. Schön, U.B. Göbel, In situ identification of nocardioform actinomycetes in activated sludge using fluorescent rRNA-targeted oligonucleotide probes, Microbiology 144 (1998) 249–259.
[6] R. J. Seviour, T. Mino, M. Onuki, The microbiology of biological phosphorus removal in activated sludge systems, Fems. Microbiol. Rev. 27 (2003) 99–127.
[7] A. Boudemagh, H. Ali-Khodja, M.F. Beldjoudi, K. Bensouici, M. Kitouni, A. Boulahrouf, M. Mahboul, H. Zaaout, N. Larkem, Biodegradation of methanol in a batch reactor by a microbial flora isolated from a wastewater treatment plant sludge at El-Menia in Constantine, Fresenius Env Bull. 15(12b) (2006) 1590–1594.
[8] S. Silini, Contribution à l'étude de la biodégradation de la méthyléthylcétone en réacteur batch par les actinomycètes isolés à partir des boues activées de la station d'épuration d'El Atmania (Contribution to the study of methyl ethyl ketone biodegradation in batch reactors by actinomycetes isolated from activated sludge at the wastewater treatment plant of El Atmania), Dissertation, Université-Mentouri Constantine, 2012.
[9] S.T. Williams, T. Cross, Actinomycetes, in: C. Booth (Ed.), Methods in Microbiology, vol. 4, Academic Press, London, 1971, pp. 295–334.
[10] F. Vandermesse, Capacité d'une communauté microbienne mixte à dégrader la MEK en réacteur batch, en vue de l'optimisation d'un bioréacteur (Capacity of a mixed microbial community to degrade MEK in a batch reactor for optimizing a bioractor), Dissertation, Université Catholique de Louvain, 1996.

[11] M.F. Beldjoudi, Biodégradation du Méthanol en réacteur batch, influence de la présence d'éthanol et des conditions de culture initiales (Biodegradation of methanol in a batch reactor, influence of the presence of ethanol and of the initial culture conditions), Dissertation, Université Mentouri-Constantine, 2004.
[12] H. Ali-Khodja, M.F. Beldjoudi, Biodégradation du méthanol en réacteur batch, influence de la présence de l'éthanol et des conditions de culture initiales, J. Soc. Alg. Chim. 15(2) (2005) 251–261.
[13] M.L. Schuler, F. Kargi, Bioprocess Engineering: Basic concepts, Edition Prentice Hall PTR, Englewood Cliffs, NJ, 1992.
[14] B. Zaitlin, S.B. Watson, J. Ridal, T. Satchwill, D. Parkinson, Actinomycetes in Lake Ontario: Habitats and geosmin and MIB production, Res. J. Can. 95(2) (2003) 113–118.
[15] S.T. Williams, F.L. Davies, Use of antibiotics for selective isolation and enumeration of actinomycetes in soil, J. Gen. Microbiol. 38 (1965) 251–261.
[16] H. Lechevalier, A practical guide to generic identification of actinomycetes, in: Bergey's Manual of Determinative Bacteriology, ninth ed., Williams and Wilkins, Baltimore, MA, 1994, pp. 611–618.
[17] S. Slim, Purification et Caractérisation de Biomolécules à partir de microorganismes nouvellement isolés et identifiés (Purification and characterization of biomolecules from newly isolated and identified microorganisms), Dissertation, Institut National Polytechnique de Toulouse 2010.
[18] E.B. Shirling, D. Gottlieb, Methods for characterization of *Streptomyces* species, Inter. J. Syst. bacteriol. 16 (1966) 313–340.
[19] L. Hilali, A. Khatabi, N. Nassrallah, A. Malki, C. Finance, Isolement des nouvelles souches d'*actinomycetales* productrices de substances antifongiques à partir du milieu naturel marocain (Isolation of new strains of actinomycetales producing antifungal substances from the Moroccan natural environment), Rev. Biol. Biotech. 2 (2002) 49–53.
[20] J.A. Salas, C. Hardisson, Sugar uptae during germination of *Streptomyces* antibioticus spores, J. Gen. Microbiol. 125 (1981) 25–31.
[21] J. Soliveri, A. Mendoza, M.E. Arias, Effect of different nutrients on the production of polyene antibiotics PA-5 and PA-7 by *Streptoverticillium* sp 43/16 in chemically defined media, Appl. Microbiol. Biotechnol. 28 (1988) 254–257.
[22] M. Takizawa, R.R. Colwell, R.T. Hill, Isolation and diversity of actinomycetes in the Chesapeake Bay, Appl. Environ. Microbiol. 59 (1993) 997–1002.
[23] S.I. Suzuki, T. Okuda, S. Komatsubara, Selective isolation and distribution of *Sporichthya* strains in soil, Appl. Env. Microbiol. 65(5) (1999) 1930–1935.
[24] C. Wu-Chung, L. Tzu-Yu, Compounds interaction on the biodegradation of acetone and methyl ethyl ketone mixture in a composite bead biofilter, Bioresource Technol. 101 (2010) 126–130.
[25] J.R. Dojlido, Investigations of Biodegradability and Toxicity of Organic Compounds, US Environmental Protection Agency, Washington, DC, 1979.
[26] J.J. Delfino, C.J. Miles, Aerobic and anaerobic degradation of organic contaminants in Florida groundwater, Soil and Crop Sci. Soc. Fl. 44 (1985) 9–14.
[27] J.R. Dojlido, Testing of biodegradability and toxicity of organic compounds, in: Polish/US Symposium on Wastewater Treatment and Sludge Disposal, 10–12 February 1976, US Environmental Protection Agency, Cincinnati, OH, vol. II, 1977, pp. 112–131.
[28] S.T. Williams, R. Locci, A. Beswick, D.I. Kurtboke, V.D. Kuznetsov, F.J. Le Monnier, P.F. Long, K.A. Maycroft, R.A. Palma, B. Petrolini, S. Quaroni, J.I. Todd, M. West, Detection and identification of novel actinomycetes, Microbiology 144 (1993) 653–656.
[29] R. Balagurunathan, L. Xu, C. Jiang, Diversity of Actinomycetes from south India and south China, Actinomycetes 4(3) (1996) 89–94.
[30] J.R. Nodwell, R. Losick, Purification of an extracellular signaling molecule involved in production of aerial mycelium by *Streptomyces coelicolor*, J. Bacteriol. 180(5) (1998) 1334–1337.
[31] S. Myadoh, N. Tsuchizaki, J. Ishikawa, K. Hotta, Digital Atlas of Actinomycetes. The Society of Actinomycetes, Tokyo, Japan, 2002. Available from: <http://www.nih.go.jp/saj/DigitalAtlas>.
[32] P. Juteau, R. Larocque, D. Rho, A. LeDuy, Analysis of the relative abundance of different types of bacteria capable of toluene degradation in a compost biofilter, Appl. Microbiol. Biotechnol. 52 (1999) 863–868.
[33] N. Khammar, L. Malhautier, V. Degrange, R. Lensi, J.J. Godon, J.L. Fanlo, Link between spatial structure of microbial communities and degradation of a complex mixture of volatile organic compounds in peat biofilters, J. Appl. Microbiol. 98 (2005) 476–490.
[34] B. Sandrine, Traitement d'effluents gazeux chargés en composés organiques volatils par oxydation biologique: Approche écologique des communautés microbiennes (Treatment of gaseous effluents containing volatile organic compounds by biological oxidation: Ecological approach of microbial communities), Dissertation, Université Claude Bernard de Lyon, 2005.
[35] R. Smita, B.V. Babu, Experimental studies and kinetic modeling for removal of methyl ethyl ketone using biofiltration, Bioresource Technol. 100 (2009) 3855–3861.