Water Availability Is a Critical Determinant of a Population Shift from *Proteobacteria* to *Actinobacteria* during Start-Up Operation of Mesophilic Fed-Batch Composting

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The effects of water availability defined as water activity (\(a_w\)) or matric water potential (\(\psi_m\)) on microbial community dynamics during the start-up operation of mesophilic fed-batch composting (FBC) of household biowaste were studied using commercially available personal composters. The community changes were monitored for 2 months by direct cell counting, quinone profiling, and 16S rRNA gene sequencing of culturable predominant bacteria. The \(a_w\) level lowered linearly with operation time and reached around 0.95 at the end of operation. During the steady-state period, \(a_w\) or \(\psi_m\) had a strong positive correlation with moisture content and ubiquinone content and negative correlation with pH, electric conductivity, and partially saturated menaquinone content. Results of 16S rRNA gene-based phylogenetic identification of the predominant isolates as well as of culture-independent quinone profiling indicated that a drastic population change from ubiquinone-containing members of *Proteobacteria* to *Actinobacteria* took place during the overall period of operation. Most of the actinobacterial isolates grew on nutrient agar with an \(a_w\) of less than 0.98, whereas none of the proteobacterial isolates did. These results suggest that water availability is an important determinant of the population shift from the *Proteobacteria* to *Actinobacteria* during the start-up operation of mesophilic fed-batch garbage composting.

**Key words:** compost, fed-batch composting, water availability, microbial community

The fed-batch composting (FBC) process is characterized by the repeated loading of biowaste without the removal of converted end products. This process provides a good model of microbial ecology in terms of microbial population dynamics during the biodegradation of solid organic substances. Practically, as for example in Japan, FBC using commercially available small-scale composters is being applied for daily treatment of household garbage. Such FBC reactors have a lower and narrower range of process temperatures than the traditional batch composting process\(^{29}\), which generally proceeds through four thermal stages\(^{6}\). Therefore, there may be a need to consider a characteristic microbiological basis for the mesophilic FBC process different from that for the conventional composting system. In recent years, the FBC process for the biotreatment of garbage and food waste has been studied from a microbiological as well as kinetic point of view\(^{3,12-14,17,22-27,31}\).

In previous studies on mesophilic FBC reactors for garbage treatment, we found that a population shift from members of the phylum *Proteobacteria* to those of the phylum *Actinobacteria* took place during the start-up period of operation\(^{12,25,29}\). Thus, we have concluded that the domination of *Actinobacteria* under steady-state conditions is a characteristic feature of the mesophilic FBC process. There is general agreement that actinobacterial species are common members of microbial populations in conventional composting systems, where temperature and substrate availability are regarded as the major factors affecting community dynamics. In the typical batch-composting process, the cooling and maturation stages have a more complex bacterial community with actinobacterial populations than the preceding thermophilic phase\(^ {15,24}\). In the mesophilic FBC process, however, it is necessary to take other determinants

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into consideration, because the process persists under relatively constant conditions with respect to temperature and nutrient availability.

Moisture is an additional critical ecological determinant in the FBC process as well as in the conventional batch composting system. It has been shown that the solid waste-compost mixture (SCM) in FBC reactors operating under steady-state conditions has 30–40% moisture, at which the microorganisms present exhibited the highest protease activity. Biologically, water availability is more critical than moisture. A parameter of water availability in a given substrate is water activity ($a_w$), which is defined as 1/100th of the relative humidity (%R.H.). A modification of water activity is matric water potential ($\psi_m$), which is given by the following formula: $\psi_m = (RT/V_a) \ln a_w$, where $R$ is the universal gas constant (8.314 J mol$^{-1}$ K$^{-1}$), $T$ is the temperature in °K, and $V_a$ is the partial molar volume of water (18 ml mol$^{-1}$). In the field of food microbiology, water availability, especially $a_w$, has received extensive study in connection with food spoilage and protection. On the other hand, few studies have been conducted on water availability as an ecological determinant in composting processes.

The main purpose of this study was therefore to elucidate the significance of water availability as an important factor affecting microbial population dynamics during the mesophilic FBC process for garbage treatment. Herein we report that members of Actinobacteria are much more tolerant to low $a_w$ than those of Proteobacteria, and this is a plausible reason why the former group of bacteria becomes predominant in the FBC process, in which the $a_w$ level of the SCM becomes low under steady-state conditions.

Materials and Methods

Operation of reactors and sample collection

Two types of commercial electric composters, a SANYO model SMS-K2 (SANYO Electric Co., Moriguchi, Japan) and a Yanmar model R5 (Yanmar Co., Osaka, Japan), were used. The reactors have a working volume of 32–33 L and contained 18–20 L of fresh wood chips as the solid matrix at the start of operation. The wood chips used were from the respective manufacturers. Detailed information about the reactors has been given previously. The biowaste used for composting was collected daily from a restaurant of Toyohashi University of Technology. The average composition of the biowaste on a wet weight basis was as follows: dark-green vegetables, 27%; yellowish-green vegetables, 39%; citrus and fruits, 12%; root crops, 8%; leftover rice and bread crumbs, 7%; fish and meat, 7%. The pH and moisture content (%) of the biowaste were 6.7 and 77 on average, respectively. The biowaste was cut into pieces of less than 3 cm square if needed, and added manually to the reactors. Both types of reactors were operated for 2 months with a 24-h-fed-batch cycle at a waste-loading rate of 0.5 kg, 0.7 kg, and 0.9 kg (wet wt) · day$^{-1}$, which are designated herein as runs A, B, and C, respectively, to study the effects of the waste-loading rate on water availability. Every hour in a batch cycle and just after waste addition, an impeller in the reactor automatically rotated to mix the SCM for 5 min. This was the only way to aerate the SCM. During the overall period of operation, the reactor was settled in an incubator room at 22°C and 50% humidity. SCM samples (5 g wet weight each) were collected from three different points of the core of the reactor at the end of each batch cycle. The samples were combined and screened by stainless steel sieve with a 5-mm mesh to remove large pieces of waste. Then, the samples were immediately subjected to physicochemical and microbiological analyses. Samples for quinone analysis were stored at −30°C until used.

Physicochemical analyses

All physicochemical analyses were performed at the end of each batch cycle. The mass reduction rate was determined by comparative measurement of the increase in the weight of the reactor and the cumulative weight of added biowaste. The weight of the reactor was measured directly on a spring balance as reported previously. The moisture content of the SCM was determined by measuring the decrease in weight after drying at 110°C for 24 h. The pH of the SCM sample was measured for its suspension 10-fold diluted with distilled water. The core temperature was measured with a digital thermometer at a depth of 8 cm in each reactor. Conductivity was measured using a portable conductivity meter. Water activity was measured using an AW SPRINT HT-500 water activity analyzer (Novasina, Pfäffikon, Switzerland) according to the manufacturer’s instructions. The $a_w$ values thus obtained were converted to $\psi_m$ values (MPa) according to the formula noted above.

Quinone analysis

SCM samples or microbial cultures were washed twice with phosphate buffer (pH 7.0) containing 1 mM ferricyanide, pellet by centrifugation at 12,600 × g for 10 min, and directly subjected to quinone extraction. Microorganisms associated with biowaste samples were released into the above-noted phosphate buffer by washing the biowaste and centrifuging at 1,500 × g for 5 min. Then, the biomass in the
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suspensions was harvested by centrifugation at 12,600×g for 10 min and subjected to quinone analysis. Quinones were extracted with an organic solvent mixture and fractionated into menaquinone and ubiquinone fractions using Sep-Pak Vac silica gel cartridges (Waters, Milford, MA, USA). Quinone components of each fraction were quantified and identified by reverse-phase HPLC and photodiode array detection with external standards. Detailed information on these analytical procedures has been given previously[16,31,36]. Ubiquinones, menaquinones, and plastoquinones with n isoprene units in their side chain were abbreviated as Q-n, MK-n, and PQ-n, respectively. Partially hydrogenated ubiquinones and menaquinones were expressed as Q-n(Hx) and MK-n(Hx), respectively, where x indicated the number of hydrogen atoms saturating the side chain. Phyloquinone (vitamin K1) was abbreviated as K1.

Direct cell counting

For cell counting, 1 g (wet wt) of SCM sample was sonicated for 90 sec (20 kHz; output power 50 W) and diluted with filter-sterilized phosphate-buffered saline (PBS). Aliquots (10–50 µl) of these diluted samples were taken and used for direct cell counting. Direct total and viable bacterial counts were measured by epifluorescence microscopy with ethidium bromide staining (EtBr) or SYBR Green II and with a Molecular Probes LIVE/DEAD BacLight Viability kit (Invitrogen Corp., Carlsbad, USA), respectively, as described previously[26,35]. Metabolically active cells were stained with a fluorescent redox dye, 5-cyano-2,3-ditolyl tetrazolium chloride (CTC), as previously reported[35]. Among CTC-positive bacteria, gram-positive bacteria were detected by post-extracting with acetone[34]. Stained specimens were observed under an Olympus BX-50 epifluorescence microscope equipped with a Flovel FD-120M digital CCD camera (Flovel Co., Tachikawa, Japan). The number of stained cells was counted using the image analysis program WINROOF (Flovel).

Plate counting and isolating

SCM samples were suspended in 9 volumes of phosphate-buffered saline and homogenated or sonicated for 90 sec to disperse microbial cells. The dispersed samples were decimally diluted with the same buffer, and 50 µl aliquots of appropriate dilutions were used for plating. Plate counts of aerobic chemoorganotrophic bacteria in SCM samples were measured by using PBYG agar medium as reported previously[15,26]. Inoculated plates were incubated in an air incubator at 30°C for 2–3 weeks before the counting of CFU. Colonies recovered on the PBYG plates from SCM samples were randomly selected and purified by repeatedly streaking agar plates. All isolates were maintained on PBYG agar slants[27] and subcultured every 3 months.

Growth tests at different levels of αw

All strains of the aerobic chemoorganotrophic bacteria isolated as noted above (strains TUT1300 to TUT1396) were tested for growth at different levels of αw. In addition, bacterial strains isolated previously from FBC systems (strains TUT1003 to TUT1096 and TUT1200 to TUT1296) were used. For this test, PBYG agar plates having different levels of αw were prepared by mixing with different concentrations of PEG 300 solution. For instance, the medium containing 0, 10, and 20% PEG 300 (w/vol) had an αw of 0.999, 0.985, and 0.973, respectively, as measured with the aforementioned water activity analyzer. Cells grown in PBYG broth as the preculture were streaked onto the agar plates with an αw of 0.999–0.940, and incubated at 30°C. Isolates showing visible colony formation within 4 weeks of incubation were regarded as being positive for growth.

16S rRNA gene sequencing and phylogenetic analysis

16S rRNA gene fragments from the cell lysate were PCR-amplified with bacterial consensus primers 1F1 (27f) and rP1 (1525r) as described previously[9]. The PCR products were separated by agarose gel electrophoresis, purified with a GENECLEAN kit (Qbiogene, Inc., Irvine, USA), and then sequenced using a SequiTherm Long Read cycle-sequencing kit (Ep incarcerated Technologies, Madison, USA), before being analyzed with an Amersham-Phannacia ALFexpress DNA sequencer. Sequence data were compiled with the GENETYX-MAC program (GENETYX Corp., Tokyo, Japan) and subjected to a homology search in the Ribosomal Database Project (RDP) to ascertain the phylogenetic positions of isolates. Multiple alignments of sequences and calculation of the nucleotide substitution rate (Ksub) with Kimura’s two-parameter model[9] were performed using the CLUSTAL W program[32]. A distance matrix tree was constructed by the neighbor-joining method[30], and the topology of the tree was evaluated by bootstrapping with 1,000 trials[4]. Alignment positions with gaps were excluded from the calculations.

Nucleotide database accession numbers

The 16S rRNA gene sequences reported in this study have been deposited under DDBJ accession numbers AB188208 to AB188225, and AB308438 to AB308445.
Results

Changes in water availability and other parameters

The two types of FBC reactor were operated for 2 months at different waste-loading rates, as shown herein as runs A, B, and C. The mass reduction rate per day in the reactors fluctuated between 75 and 98% during the first 2 weeks and became stable thereafter, being 95, 94, and 90% on average in runs A, B, and C, respectively (data not shown). In this respect, the reactors might be regarded to be under steady-state conditions after 2 weeks of operation. As shown in Fig. 1, similar changes in other physicochemical parameters were found regardless of the type of reactor and the waste-loading rate. The core temperature fluctuated between 31 and 45°C with self-heating (Fig. 1a), indicating that the composters were kept under mesophilic conditions. The pH of the SCM decreased to 5.3 to 6.2 early on and then increased gradually during the overall period of composting, reaching 9.5–9.7 after 2 months of operation (Fig. 1b). The moisture content was relatively high for 1 month from the start of waste delivery but decreased to 30 to 40% at the end of operation (Fig. 1c). These physicochemical changes are typical of the mesophilic FBC process during the start-up period as well documented[20]. The $a_w$ decreased linearly with time and reached a value of 0.950 to 0.955 at the end of operation (Fig. 1d), corresponding to a $\psi_m$ level of -6.53 to -7.23 MPa. However, $a_w$ greatly fluctuated during the latter period of operation. In particular, this was the case in run C.

This observation might result from greater differences in $a_w$ between the SCM under steady-state conditions and the fresh biowaste added. When conductivity was continuously monitored in run C, it was found to have increased linearly with time and reached 3.5 mS cm$^{-1}$ after 60 days of operation (data not shown). Similar conductivity values (3.2–3.4 mS cm$^{-1}$) were obtained at the end of operation in runs A and B.

All data on $a_w$ and other physicochemical parameters, except those obtained during the first 2 weeks when the performance of the reactors remained unstable, were statistically analyzed to elucidate their correlations under steady-state conditions. In all runs in each reactor, $a_w$ and $\psi_m$ had a strong positive correlation with moisture content ($r^2=0.402–0.856$) and a strong negative correlation with pH ($r^2=0.388–0.760$), all of which are statistically significant ($P<0.01$, n=46). There was also a significant negative correlation between $a_w$ or $\psi_m$ and conductivity ($r^2=0.470–0.514$). These results suggested that the decrease in water availability during the composting resulted from the accumulation of mineral salts that were daily introduced with the biowaste into the reactors.

Since no significant differences were noted in physicochemical and microbiological data between the two reactors, only results obtained with the SANYO reactor will be described below.

Succession of bacterial populations

In all runs, the total bacterial count increased markedly from the beginning and reached the order of $10^{11}$ cells g$^{-1}$ (dry wt) of the SCM during the first 3 weeks of operation. Thereafter, the total count became stable between $3.3\times10^{11}$ and $5.7\times10^{11}$ g$^{-1}$ (dry wt). Similar total counts have been recorded for commercial FBC reactors previously[23,26]. The direct viable count measured with a LIVE/DEAD BacLight kit accounted for 75–90% of the total count during the first 2 weeks and 55–65% thereafter. The CTC count was also 60–85% of the total population early on but dropped sharply to 8–20% under steady-state conditions. Most of the CTC-positive cells found at the steady-state stage were not affected by acetone treatment. This suggested that most of the metabolically active bacteria at the steady-state stage were gram positives, as it has been shown that CTC-positive gram-positive bacteria are not decolorized with acetone[24]. The culturability as shown by the plate count/total count ratio was less than 20% during the first 2 weeks but increased to approximately 50% thereafter.

As noted above, changes in the plate count did not always match those in the LIVE/DEAD BacLight or CTC count.
Also, it was curious that the CTC count was lower than the plate count under steady-state conditions. The reason for this was unknown. It is clearly necessary to check whether the CTC-staining method used, optimized for activated sludge populations\(^{19}\), is applicable to compost environments.

**Succession of quinone profiles**

In parallel with the total bacterial count, the total quinone content of the SCM sharply increased during the first 3 weeks in all runs and thereafter steadied at between 190 and 280 nmol g\(^{-1}\) (dry wt). By assuming that 1 nmol of total respiratory quinone corresponds to 2.1×10\(^6\) cells of bacteria in soil and compost\(^{11}\), the total count predicted based on the quinone content is 4.0–5.9×10\(^{11}\) cells g\(^{-1}\). These values are similar to the total counts actually measured. Changes in concentrations of ubiquinones, unsaturated menaquinones, and partially saturated menaquinones in runs A, B, and C are shown in Fig. 2. In all runs, ubiquinones predominated early in the process but declined with time. In contrast, partially saturated menaquinones increased gradually with time and predominated during the latter half of operation, accounting for 42–57 mol\% at the end of operation. A regression analysis of the quinone dynamics revealed that the day on which the domination by ubiquinones and partially saturated menaquinones was reversed was 44 in run A, 31 in run B, and 32 in run C (as indicated by arrows in Fig. 2). The SCM at these points had an \(a_w\) level of 0.968–0.971 (average of 3 runs, 0.970). Unsaturated menaquinones accounted for 20 to 32% of the total during the overall period, except the first 3–5 days. In view of these results, together with the available information on quinone systems of microorganisms\(^{10}\), it was evident that a population shift from ubiquinone-containing Proteobacteria to Actinobacteria took place during the composting of the household biowaste.

Detailed information about the quinone profiles in the 3 runs of the FBC reactor at the end of operation compared to that of the microorganisms attached to the biowaste are given in Table 1. In all the runs, the most abundant quinone species detected were MK-8(H\(_2\)) (18–27 mol\%), and additional 9 homologs of partially hydrogenated menaquinones were detected. Other quinone species accounting for more than 10 mol\% were MK-7 and MK-8 in run C, Q-8 in run A, and Q-10 in runs A and C. These data clearly indicate that bacteria containing different homologs of partially saturated menaquinones, i.e., certain members of Actinobacteria\(^{10}\), predominated at the acclimated stage. In contrast, the quinone composition of the biowaste-associated microorganisms was characterized by an overwhelming majority of ubiquinones with Q-8 as the most abundant homolog. A significant proportion of PQ-9 and K\(_1\) was also detected in this fraction. These photosynthetic quinones might be derived from the vegetable waste carried over into samples.

Relationships between the amount of ubiquinone, unsaturated menaquinone, or partially saturated menaquinone, and physico-chemical parameters were statistically analyzed. As shown in Table 2, the ubiquinone or unsaturated menaquinone content had a strong positive correlation to moisture content and water availability and a negative correlation to pH and conductivity. The reverse was the case for partially hydrogenated menaquinones.

**Phylogenetic composition and growth response to different \(a_w\) levels of isolates**

The dominant bacteria in the run B reactor operating on days 3 and 56 were isolated by the quantitative agar-plating method. A total of 94 isolates thus obtained were phylogenetically analyzed by partial 16S rRNA gene sequencing.

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**Fig. 2.** Changes in quinone profiles during fed-batch composting of household garbage in runs A (a), B (b), and C (c). Symbols: triangles, ubiquinones; closed circles, unsaturated menaquinones; open circles, partially saturated menaquinones. Plots for ubiquinones and partially saturated menaquinones are lined up on the basis of regression analyses. Arrows indicate the points at which the amounts of ubiquinones and partially saturated menaquinones became equal.
Table 1. Quinone composition of SCM in different runs of the FBC reactor and the biowaste used

<table>
<thead>
<tr>
<th>Quinone species (and phylogenetic groups as possible major sources)</th>
<th>Quinone content (mol%)</th>
<th>Reactor in run</th>
<th>Biowaste*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q-n (Alpha-, Beta-, and Gammaproteobacteria and mitochondria)</td>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Q-7</td>
<td>0.1</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Q-8</td>
<td>12.5</td>
<td>6.9</td>
<td>6.2</td>
</tr>
<tr>
<td>Q-9</td>
<td>3.5</td>
<td>1.9</td>
<td>2.3</td>
</tr>
<tr>
<td>Q-10</td>
<td>13.2</td>
<td>9.4</td>
<td>11.5</td>
</tr>
<tr>
<td>Q-11</td>
<td>0.6</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Q-10(H2)</td>
<td>0.4</td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td>PQ and K1 (chloroplasts)</td>
<td></td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>PQ-9</td>
<td></td>
<td>0.6</td>
<td>1.0</td>
</tr>
<tr>
<td>K1</td>
<td></td>
<td>0.6</td>
<td>1.0</td>
</tr>
<tr>
<td>MK-n (Actinobacteria, Bacteroidetes, Firmicutes)</td>
<td></td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>MK-6</td>
<td>4.9</td>
<td>3.0</td>
<td>1.3</td>
</tr>
<tr>
<td>MK-7</td>
<td>8.3</td>
<td>8.1</td>
<td>11.5</td>
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<td>6.9</td>
<td>7.2</td>
<td>10.3</td>
</tr>
<tr>
<td>MK-9</td>
<td>2.8</td>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td>MK-10</td>
<td>1.9</td>
<td>2.2</td>
<td>1.1</td>
</tr>
<tr>
<td>MK-11</td>
<td>0.7</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>MK-12</td>
<td>0.5</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>MK-n(H2) (Actinobacteria)</td>
<td></td>
<td>0.5</td>
<td>0.2</td>
</tr>
<tr>
<td>MK-7(H2)</td>
<td>5.2</td>
<td>5.1</td>
<td>2.9</td>
</tr>
<tr>
<td>MK-7(H4)</td>
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<td>1.0</td>
<td>0.6</td>
</tr>
<tr>
<td>MK-8(H2)</td>
<td>3.4</td>
<td>5.5</td>
<td>4.0</td>
</tr>
<tr>
<td>MK-8(H4)</td>
<td>18.0</td>
<td>27.4</td>
<td>26.7</td>
</tr>
<tr>
<td>MK-8(H6)</td>
<td>0.7</td>
<td>1.2</td>
<td>0.3</td>
</tr>
<tr>
<td>MK-9(H2)</td>
<td>4.3</td>
<td>6.2</td>
<td>5.5</td>
</tr>
<tr>
<td>MK-9(H4)</td>
<td>4.0</td>
<td>3.8</td>
<td>5.5</td>
</tr>
<tr>
<td>MK-9(H6)</td>
<td>2.8</td>
<td>4.3</td>
<td>2.0</td>
</tr>
<tr>
<td>MK-9(H8)</td>
<td>3.5</td>
<td>2.7</td>
<td>4.6</td>
</tr>
<tr>
<td>MK-10(H2)</td>
<td>0.3</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Others</td>
<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
</tr>
</tbody>
</table>

* Data on day 56.

** Average and standard deviations of four different determinations (on days 3, 14, 28, and 56).

(660–720 bp) and quinone profiling. Nearly complete sequences of 16S rRNA genes (1,450–1,520 bp) were also determined for representatives of the isolates. The phylogenetic composition of these culturable bacteria at the phylum and class levels as determined by the RDP-II homology search is summarized in Fig. 3. Most of the strains isolated on day 3 were identified as members of the ubiquinone-containing Proteobacteria, especially those of the Gammaproteobacteria. The RDP-II search showed that the major genera to which more than 10% of the isolates on day 3 were assigned were Acinetobacter (13%), Enterobacter (19%), Mesorhizobium (11%), Paracoccus (15%), and Pseudomonas (11%) (see also Fig. 5). On the other hand, the majority of the bacteria isolated on day 56 were assigned to members of Actinobacteria with partially saturated menaquinones, and a significant proportion was represented by members of the Firmicutes. The major genera (>10%) found on day 56 were Bacillus (22%), Cellulosimicrobium (11%), Ornithinococcus (16%), and Rhodococcus (10%), which contained MK-7, MK-9(H4), MK-8(H4), and MK8(H2) as the major quinones, respectively.

All of the isolates were tested for growth on nutrient agar
Table 2. Correlations between ubiquinone, menaquinone or partially saturated menaquinone contents (mol%) and physicochemical parameters obtained in the SANYO reactor

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Correlation coefficient (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Q-n</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>0.2373</td>
</tr>
<tr>
<td>pH</td>
<td>-0.9108a</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>0.6885a</td>
</tr>
<tr>
<td>Water activity</td>
<td>0.7488a</td>
</tr>
<tr>
<td>Matric potential (MPa)</td>
<td>0.7488a</td>
</tr>
<tr>
<td>Conductivity (µS cm⁻¹)</td>
<td>-0.8255a</td>
</tr>
</tbody>
</table>

* Significant at P<0.01 (n=20, except for conductivity [n=7]).
  
* Significant at P<0.05 (n=7).

media with different αₚ levels (Fig. 4). Most of the actinobacterial isolates were able to grow at an αₚ of 0.974, and 40% still grew at an αₚ of 0.965. On the other hand, the growth response of the proteobacterial isolates sharply declined with decreasing αₚ, and none of them grew at an αₚ of less than 0.974. The Firmicutes isolates were much more tolerant than the proteobacterial isolates but were more sensitive than the actinobacterial isolates to low αₚ.

To confirm the universality of the aforementioned phenomena, several bacterial strains previously isolated from different FBC processes and identified with a wide variety of genera of Actinobacteria, Firmicutes, and Proteobacteria²²,²⁵ were also tested for growth response to low αₚ. Combined data on the phylogenetic positions, quinone systems, and growth response to low αₚ of these isolates are shown in Fig. 5. In accordance with the results shown in Fig. 4, almost all strains of Actinobacteria previously isolated grew at an αₚ level of 0.974, whereas the proteobacterial isolates did not. These results suggest that tolerance to an αₚ as low as 0.97 is a trait usable for classifying compost-derived bacterial strains at the phylum level.

Discussion

In this study, we have entirely confirmed our previous observations that a population shift from ubiquinone-containing Proteobacteria to Actinobacteria takes place during the start-up operation of the mesophilic FBC process²²,²³,²⁵. That is, the SCM in the FBC reactors during the early phases of operation contained ubiquinones as the major class of quinone, whereas partially hydrogenated menaquinones became predominant under steady-state conditions. Also, the predominant culturable bacteria isolated from the early stages of FBC operation were assigned to proteobacterial genera with ubiquinones such as Acinetobacter, Enterobacter, Mesorhizobium, Paracoccus, and Pseudomonas. On the other hand, the major genera found under steady-state conditions were Bacillus and those of the Actinobacteria with partially saturated menaquinones, including Cellulosimicrobium, Ornithinococcus, and Rhodococcus. The community composition thus reconstructed is consistent with the quinone profiles of the SCM itself taken at different stages of operation. Bacteria of the aforementioned genera have been isolated previously in mesophilic FBC processes using flowerpots²² and commercial composters²³, depending upon the acclimation phase. In general, most of the prokaryotes in natural environments are hardly cultivable with laboratory-used culture media⁹,²⁰, whereas a high culturability of microorganisms in FBC reactors has been shown by comparative data on direct total, LIVE/DEAD, CTC, and plate counts as reported herein and

![Fig. 3. Phylogenetic composition of aerobic heterotrophic bacteria isolated from an FBC reactor operated at a waste loading rate of 0.7 kg day⁻¹ (in run B). Data on the strains isolated on days 3 (left) and 56 (right) are shown. The phylogenetic allocation of the isolates was based on the results of an RDP-II homology search analysis.](image-url)
on these days had similar \( a_w \) levels (average, 0.970) irrespective of the waste-loading rate. This suggests that an \( a_w \) level of around 0.970 in the FBC process is the critical point at which *Actinobacteria* can replace ubiquinone-containing members of *Proteobacteria* as the major constituents of the microbial community. In fact, none of the proteobacterial strains isolated from the FBC reactors grew on culture media with an \( a_w \) of less than 0.974, whereas most of the actinobacterial isolates were able to grow at such a low \( a_w \).

It is most likely that the ability to tolerate low water availability leads *Actinobacteria* to survive and predominate during FBC under steady-state conditions. When the FBC reactors were operated for 1 yr with a 2-month interval of withdrawing excess end products, the \( a_w \) level remained relatively constant (~0.95), and members of the *Actinobacteria* and *Bacillus* continued to predominate (unpublished observations).

Since FBC reactors have an alkaline pH under steady-state conditions, the effect of pH on the microbial community structure cannot be ignored. However, almost all of the predominant culturable bacteria isolated so far from different stages of the FBC process within 60 days of operation are neutrophilic (pH optimum for growth, 6.5–7.5) (unpublished data). Therefore, pH may exert less effect on the population shift during 2 months of FBC operation, if any, compared to \( a_w \).

In the FBC processes studied herein and previously\(^{12,13,23,25}\), it is obvious that the reactors were continuously seeded with large numbers of exogenous microorganisms with daily loading of garbage. Quinone profiling has demonstrated that the overwhelming majority of these microorganisms are ubiquinone-containing members of the *Proteobacteria*. Therefore, seeding of high numbers of these ubiquinone-containing bacteria with biowaste may bring about their proliferation and predominance at the early stage of FBC. Along with operation time, however, the ubiquinone-containing proteobacteria may become unable to maintain their large populations because of a decrease in water availability. Alternatively, members of the *Actinobacteria*, which are possibly introduced in much smaller numbers with the biowaste, become more abundant under such low \( a_w \) conditions.

In conclusion, water availability is one of the most important determinants of the population dynamics in the FBC process. In conventional sequencing-batch composting processes, it is unlikely that \( a_w \) decreases with operational time, because mineral salts with converted end products are continuously removed from the process. Therefore, it seems reasonable to conclude that the population dynamics as an

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Fig. 4. Growth response to different \( a_w \) levels of the isolates belonging to *Actinobacteria* (a), *Firmicutes* (b), and *Proteobacteria* (c).

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Previously, therefore, our results of the FBC community analysis based on the culturable bacterial strains may provide relatively reliable information, although the question of why the FBC reactors under steady-state conditions gave higher plate counts than CTC counts remains unanswered.

The present study has revealed that water availability defined as \( a_w \) or \( p_f \) linearly decreases with operation time in the FBC reactors, reaching around 0.95 (as \( a_w \)) at the end of operation. Statistical analyses of different parameters recorded for the reactors have revealed that \( a_w \) had a strong positive correlation with ubiquinone content and a negative correlation with partially hydrogenated quinone content. Also, \( a_w \) had a strong positive correlation with pH and a negative correlation with conductivity. These data suggest that the decrease in water availability during FBC operation resulted from the accumulation of soluble mineral salts with operation time, and this parameter is a critical determinant of the community succession from ubiquinone-containing bacteria (i.e., *Alpha-*, *Beta-*, and *Gamma*-proteobacteria) to those with partially saturated menaquinones (i.e., *Actinobacteria*).

During the start-up period of FBC operation, the days on which the domination by ubiquinones and partially saturated menaquinones became reversed were 31 to 44, depending upon the waste-loading rate. However, the SCM
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Fig. 5. 16S rRNA gene-based neighbor-joining phylogenetic tree for the isolates from different FBC reactors. The accession numbers for 16S rRNA gene sequences are shown in parentheses just behind the organism names. The 16S rRNA sequence of *Aquific pyrophilus* (M) was used as the outgroup to root the tree. Scale bar: 2% nucleotide substitution. The nodes supported by a bootstrap value of more than 800 (1,000 resamplings) are shown by open circles. Quinone systems and growth response to different *a_w* levels of the isolates are shown on the right side (+, positive; -, negative). The strains with TUT numbers of 1000–1015, 1200–1250, and 1300–1394 (in bold face) were isolated from FBC reactors by Hiraishi et al.\(^1\), by Narihiro et al.\(^2\), and in this study, respectively.
effect of the reduction of $a_n$ is a characteristic feature of the FBC process. The significant difference in tolerance for low water availability between members of *Proteobacteria* and *Actinobacteria* is possibly important in the microbial ecology of these bacteria in different natural environments.

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