Influence of Dissolved Oxygen Concentration Change on Musty Odor Production by Actinomycetes

Azusa Hashimoto\textsuperscript{1}, Kazuya Shimizu\textsuperscript{2}, Norio Sugiura\textsuperscript{3}, Motoo Utsumi\textsuperscript{3}

\textsuperscript{1}Graduate School of Life and Environmental Sciences, University of Tsukuba
\textsuperscript{2}Faculty of Life Sciences, Toyo University
\textsuperscript{3}Faculty of Life and Environmental Sciences, University of Tsukuba
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Unpleasant odor in drinking water leads to consumer complaints and economic losses.
Motivation

Influence of environmental factors

Microorganisms

Actinomycetes  Cyanobacteria

Musty odor compounds

Geosmin  MIB

As for geosmin production by actinomycetes…

- pH and temperature (Sugiura, 1989)
- Carbon sources, phosphorus concentration, and several metals (Schrander and Blevins, 2001)
Motivation

Influence of environmental factors

Microorganisms

Actinomycetes Cyanobacteria

Musty odor compounds

Geosmin MIB

As for geosmin production by actinomycetes:

- pH and temperature (Sugiura, 1989)
- Carbon sources, phosphorus concentration, and several metals (Schrander and Blevins, 2001)

Environmental factors of sediment

Growth and geosmin production by actinomycetes
Dissolved oxygen (DO) concentration change

Motivation

Thermocline is developed.

Vertical water mixing stops.

Oxygen supply to bottom layer stops.

Oxygen is consumed by degradation of organic matter.

Temperature

Low → High

Lower DO
It has not been investigated whether or not changing DO concentration affect geosmin production by actinomycetes.
Evaluate the effect of different DO concentration on growth and geosmin production by *Streptomyces coelicolor* A3(2)

**Objective**

**Preculture**
- DO 8 mg/L
  - Dark condition, 30°C, 120 rpm, 2 days

**Culture at different DO concentration**
- Initial DO concentration: 0, 0.4, 2, 4, 8 mg/L
- Temporally changing DO concentration:
  - 0 mg/L → 8 mg/L
  - Incubation time (day): 0, 1, 2, 3, 4, 5, 6
Materials and Methods

Preculture under aerobic condition (DO 8 mg/L) → Harvest and wash → Inoculation into new media
30°C, 120 rpm, 2 days

Incubation
30°C, 120 rpm, 3 days

Measurement of geosmin concentration and optical density (OD\textsubscript{450})
Materials and Methods

Preculture under aerobic condition (DO 8 mg/L)

Harvest and wash

Inoculation into new media

Effect of initial DO concentration

<table>
<thead>
<tr>
<th>DO (mg/L)</th>
<th>Purge with</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100% N₂</td>
</tr>
<tr>
<td>0.4</td>
<td>99% N₂ + 1% O₂</td>
</tr>
<tr>
<td>2</td>
<td>95% N₂ + 5% O₂</td>
</tr>
<tr>
<td>4</td>
<td>90% N₂ + 10% O₂</td>
</tr>
<tr>
<td>8</td>
<td>Air</td>
</tr>
</tbody>
</table>

Purge with mixture of O₂ and N₂

30°C, 120 rpm, 2 days
Inoculation into new media

Preculture under aerobic condition (DO 8 mg/L)
- 30°C, 120 rpm, 2 days

Harvest and wash

Inoculation into new media

Incubation
- 30°C, 120 rpm, 3 days

Measurement of geosmin concentration and optical density (OD$_{450}$)
Materials and Methods

Preculture under aerobic condition (DO 8 mg/L) → Harvest and wash → Inoculation into new media

**Effect of temporally changing DO concentration**

- **Anaerobic condition (0 mg/L) for 1 day**, and then aerobic condition (8 mg/L) for another 3 days

- **Anaerobic condition (0 mg/L) for 3 days**, and then aerobic condition (8 mg/L) for another 3 days

- **Anaerobic control (0 mg/L)**

- **Aerobic control (8 mg/L)**
Results and Discussion

Effect of initial DO concentration

![Graph showing the effect of initial DO concentration on Geosmin production and OD450 over incubation time.](image)
Results and Discussion

Effect of initial DO concentration

- Growth was not observed (○, ●).
- Logarithmic phase was observed from day 0 to day 1, and stationary phase was observed from day 1 to day 3 (●, ■, ●).
- The ratio of OD_{450} (2 and 4 mg/L) to 8 mg/L condition were 50% (●) 80% (■) on day 1, respectively.
Results and Discussion

Effect of initial DO concentration

- Geosmin was not produced (○, ∙).
- Geosmin was produced during logarithmic and early stationary phase (○, ●, ●).
Results and Discussion

Effect of initial DO concentration

- Higher DO concentration showed higher geosmin production activity of each cell during logarithmic phase (●).
- There were not significant differences in geosmin production activities of each cell during stationary phase (●, , ●).
Results and Discussion

Effect of temporally changing DO concentration

- OD$_{450}$
- Geosmin (nM)
- Geosmin (nM)/OD$_{450}$

Incubation time (day):
- 0 1 2 3 4 5 6

1 day anaerobic, 3 days aerobic condition
3 days anaerobic, 3 days aerobic condition
Aerobic control (8 mg/L)
Anaerobic control (0 mg/L)
Results and Discussion

Effect of temporally changing DO concentration

- Similar growth was observed (▲, △).
- Logarithmic phase lengthened into 2 days (▲).
- The ratio of OD$_{450}$ to aerobic control or 1 day anaerobic, 3 days aerobic condition was 60% during stationary phase (▲).

![Graph showing changes in OD$_{450}$ and Geosmin concentrations over incubation time.](image)

- ▲ 1 day anaerobic, 3 days aerobic condition
- △ 3 days anaerobic, 3 days aerobic condition
- ▲ 3 days anaerobic, 3 days aerobic condition
- △ Aerobic control (8 mg/L)
- ▲ Anaerobic control (0 mg/L)
Results and Discussion

Effect of temporally changing DO concentration

- Final geosmin amount was lower than aerobic control (▲, ▲).
- Final geosmin amount was much lower than 1 day anaerobic, 3 days aerobic condition (▲▲).

1 day anaerobic, 3 days aerobic condition
3 days anaerobic, 3 days aerobic condition
Aerobic control (8 mg/L)
Anaerobic control (0 mg/L)
Effect of temporally changing DO concentration

Results and Discussion

The ratios of geosmin production activities of each cell to aerobic control were 70% (△) and 50% (▲) on the final day, respectively.

The slope of ▲ was smaller than that of △.

1 day anaerobic, 3 days aerobic condition
3 days anaerobic, 3 days aerobic condition
Aerobic control (8 mg/L)
Anaerobic control (0 mg/L)
Conclusions

Effect of initial DO concentration

- Initial DO concentration affected growth and geosmin production by *S. coelicolor* A3(2).
- Growth and geosmin production were not observed below 0.4 mg/L condition.

Effect of temporally changing DO concentration

- Prolonged exposure to anaerobic condition resulted in depression of growth and geosmin production.
- Slopes of growth and geosmin production decreased with longer anaerobic condition.

These results indicated that *in situ* DO condition should affect growth and geosmin production by actinomycetes.
Thank you for listening.