Phenol degradation and production of PHA using phenol degradation products as substrates

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▪Goals

Phenol is found in industrial wastewater from the petroleum and chemical products industries, where coke and phenolic resins are produced. Wastewater containing phenol is toxic to living organisms and needs to be treated using chemicals.

For this reason, **the cost of treatment is higher than that of general wastewater treatment**.

Solution

In this background, we aimed to add value to wasted phenol by using it as a substrate to produce polyhydroxyalkanoic acid (PHA), a biodegradable plastic.

PHA is a biopolymer produced in the cells of microorganisms and is known as an intracellular storage material that is utilized when there is no suitable carbon source in the growing environment. It is attracting attention as an alternative to petroleum-based plastics because of its similar properties to polyethylene (PE) and polypropylene (PP) and its ability to biodegrade in the natural environment.

Photo. 1. Cokes oven[1)]

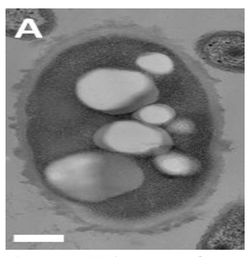


Fig. 1. PHA accumulation in *Bacillus* sp. CYR1 using phenol as a carbon source (TEM)[2)]

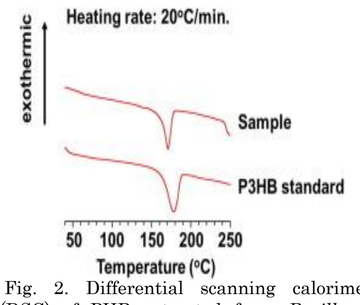
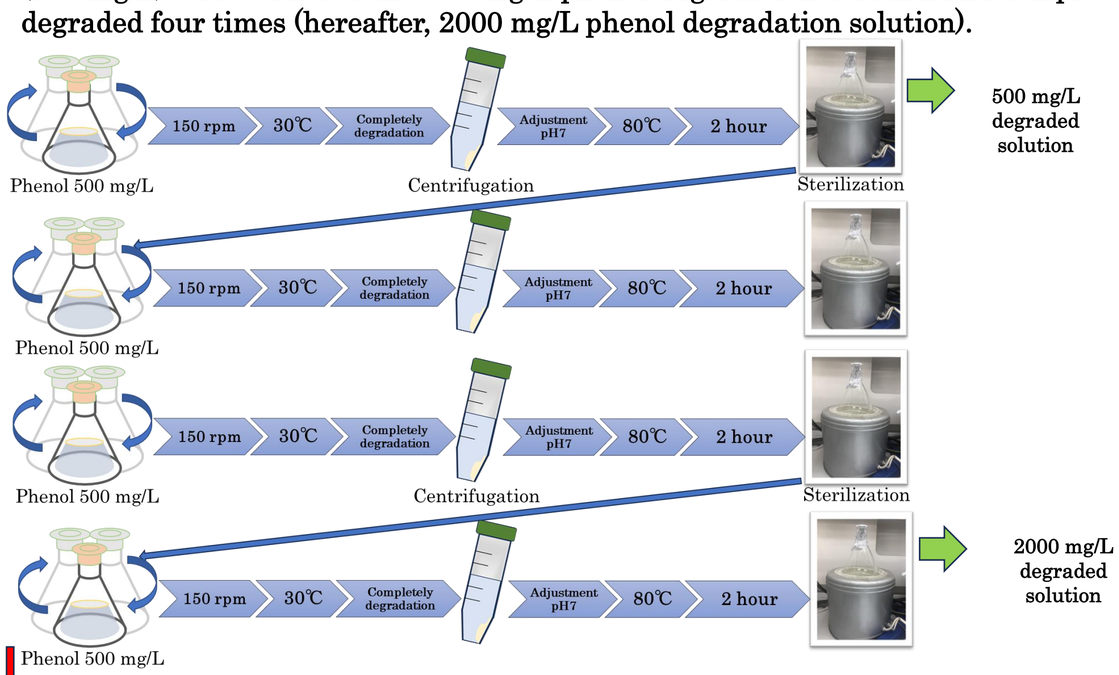


Fig. 2. Differential scanning calorimetry (DSC) of PHB extracted from *Bacillus* sp. CYR1 and standard PHB[2)]

References
1) https://e-kensin.net/news/121557.html
2) Reddy, M. Venkateswar, et al. *Bioresource Technology*, 2015, 192: 711-717.
3) Saito, K., Reddy, M. V., Sarkar, O., Kumar, A. N., Choi, D., & Chang, Y. C. Bioengineering. 10(5), 618. 2023.

▪Method

1) Phenol degradation products (liquid) obtained from the complete degradation of phenol (500 mg/L) in a single triangular flask using *Comamonas* sp. C1, a phenol-degrading bacterium (hereafter, 500 mg/L phenol degradation solution), and phenol (500 mg/L) were added to the 500 mg/L phenol degradation solution and completely degraded four times (hereafter, 2000 mg/L phenol degradation solution).



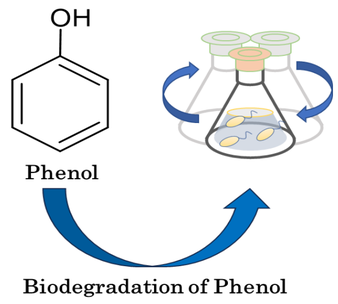
▪Experiments scheme

Selection of efficient phenol-degrading bacteria

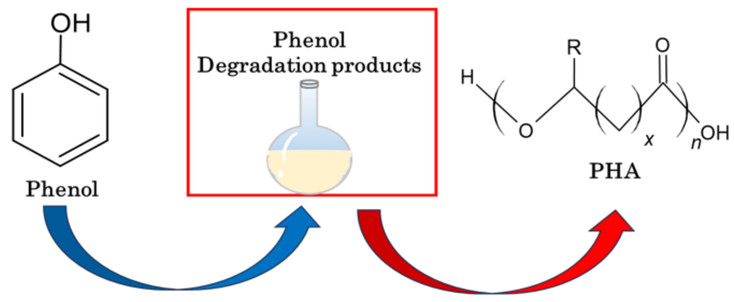
Rhodococcus erythropolis Sk-1
Collected from contaminated soil — Max : 500 mg/L, 96 h

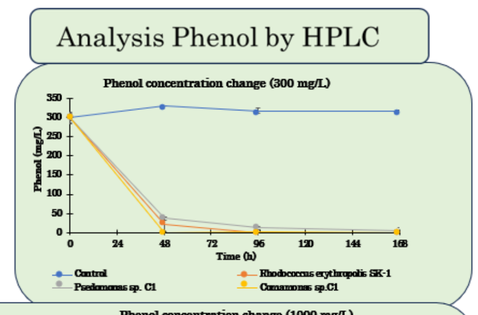
Pseudomonas sp. C1
Collected from contaminated wastewater — Max : 300 mg/L, 96 h

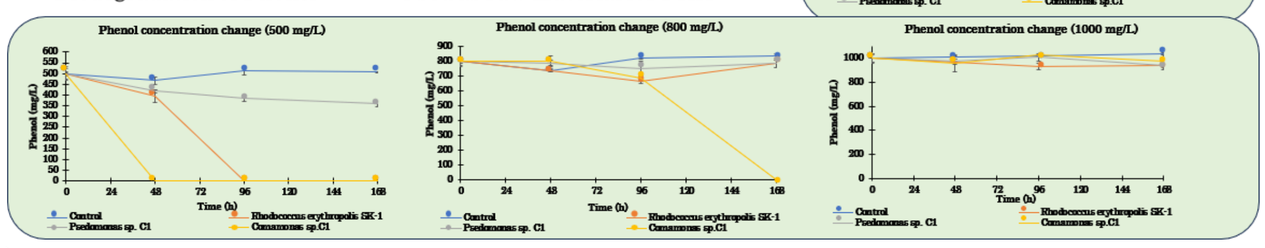
Comamonas sp. C1
Collected from contaminated wastewater — Optimum : 500 mg/L, 34 h Max : 800 mg/L



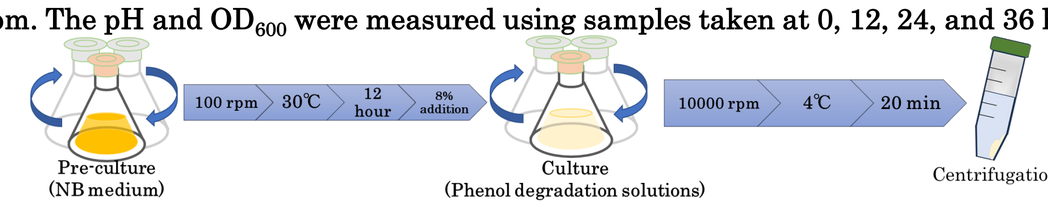
PHA production using phenol degradation products







2) PHA production experiments were conducted in a pre-culture and main-culture flow. In the pre-culture, the inoculum was inoculated into NB medium using a platinum ear and incubated at 30℃, 100 rpm, for 12 hours with shaking. In the main-culture, 8% (v/v) of the pre-culture was added to the above solution, and the incubation was carried out at 30℃, 150 rpm. The pH and OD_{600} were measured using samples taken at 0, 12, 24, and 36 hours.



3) PHA concentration was determined using the alkaline pretreatment method by Saito et al.3). For alkaline pretreatment, NaOH was added to the dried bacteria for alkaline decomposition. HCl was added to adjust the pH to 3, and the alkaline pretreatment sample was adjusted to the volume of 10 mL with ultrapure water. The prepared samples were filtered through membrane filters and used for HPLC analysis.

Photo. Dried fungi

Alkali treatment → HCl addition → Meth up to 10 mL UPW

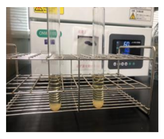


Photo. treated samples

Filtration by PTFE membrane filter → Analysis of PHA concentration by HPLC

Table 1. PHA production by *Bacillus* sp. CYR1

Sample name	CDW (mg/L)	PHA productions (mg/L)	PHA content (%)
500 mg/L degraded solution	166	9.8	6
2000 mg/L degraded solution	180	54.5	30

▪Results

Figure 3 shows the change in pH with time for the phenol degradation solution. From the results of Figure 3, it is confirmed that pH increases with time in both conditions. Figure 4 shows the change over time of OD_{600} when phenol degradation solutions are used. From the results of Figure 4, it is confirmed that OD_{600} increases in both conditions, and the maximum OD_{600} value is observed in the condition of 2000 mg/L degradation solution.

The results of PHA production by *Bacillus* sp. CYR1 using 500 mg/L and 2000 mg/L degradation solution showed that 9.8 mg/L of PHA was produced from 166 mg/L cell dry weight (6% PHA yield) in the 500 mg/L degradation solution, while 54.5 mg/L of PHA was produced from 180 mg/L cell dry weight (30.2% PHA yield) in the 2000 mg/L degradation solution.

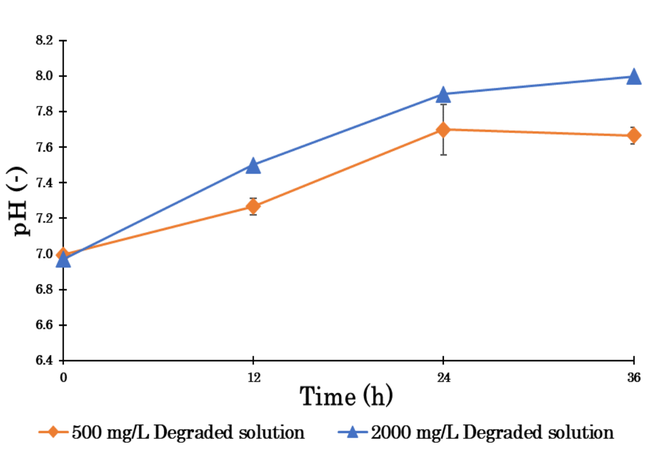


Fig. 3. Change over time of pH in PHA production by *Bacillus* sp. CYR1

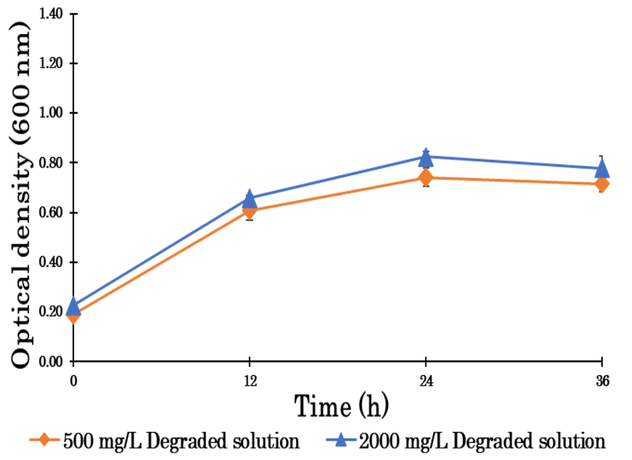


Fig. 4. Change over time of OD_{600} in PHA production by *Bacillus* sp. CYR1

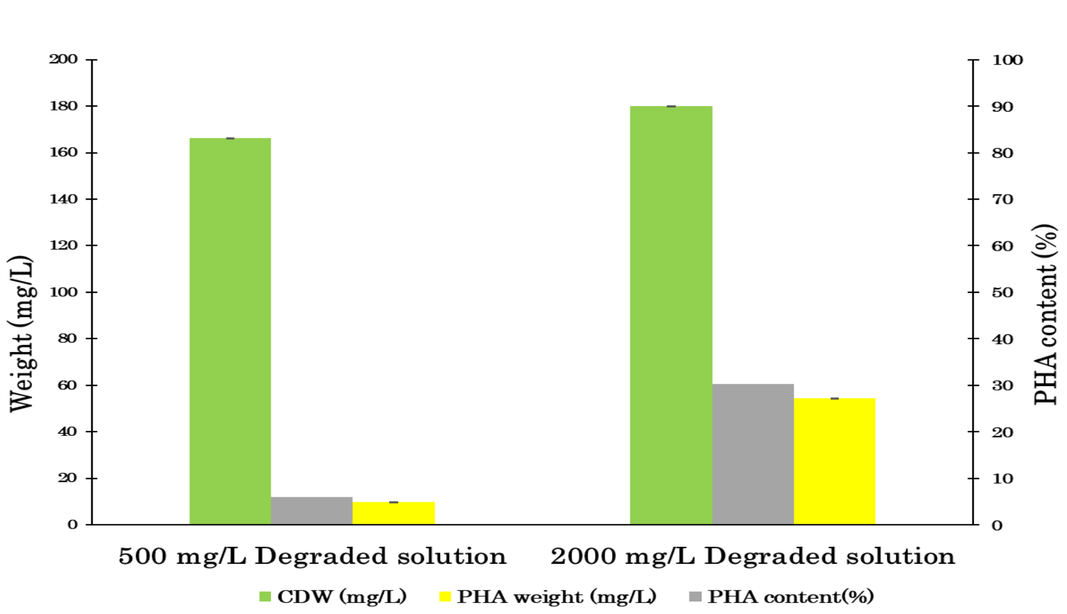


Fig. 5. PHA production by *Bacillus* sp. CYR1

Comparison with previous studies

The previous PHA production study added 0.02% (v/v) of Tween 80 as an auxiliary substrate for growth with phenol (100 mg/L). At that time, the cell dry weight reached 1.01 g/L, and the PHA production amount was 0.515 g/L, higher than this study's. The low OD_{600} in this study suggests that either the substrate concentration in the phenol degradation products is low or the addition of auxiliary growth substrates, such as Tween 80, is needed for the growth of *Bacillus* sp. CYR1.

About Phenol Degradation Products

Comamonas sp. C1, the phenol-degrading bacterium used in this study, is known to produce catechol and benzoic acid as degradation products (data not shown). In the HPLC analysis of the phenol-degrading solution (completely degraded phenol) used for PHA production in this experiment, no catechol and benzoic acid peaks were observed. Therefore, it is highly possible that the degradation products used to produce PHA are not catechol and benzoic acid, but the lower-level degradation products that have been further degraded.

Conclusions

- This experimental method allows for the accumulation of phenol degradation products, and the production of PHA using these degradation products has proven successful.
- The phenol degradation products utilized in PHA production may not include catechol or benzoic acid.
- The efficient accumulation of phenol degradation products requires further exploration.
- Cell proliferation of *Bacillus* sp. CYR1 is a crucial factor in PHA production, and the addition of substrates is necessary to support this proliferation.