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The Potential of Non-Sterilized Sewage Water as a Dilution Medium for PHA Production by *Bacillus* sp. CYR1

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INTRODUCTION & AIM

PHA (polyhydroxyalkanoate) is expected to be an environmentally friendly and biodegradable alternative to petroleum-based plastics. However, its high production cost remains a major challenge. To address this issue, sewage was considered as a low-cost raw material. Previous studies demonstrated that PHA can be produced by *Bacillus* sp. CYR1 using sterilized sewage as a dilution medium[1], but sterilizing large volumes of sewage is not practical on an industrial scale. Moreover, when only unsterilized sewage was used, no bacterial growth was observed due to the low concentration of organic matter in the sewage water [2]. Therefore, this study aimed to evaluate the potential use of unsterilized sewage as a dilution medium by comparing PHA production under sterilized sewage and unsterilized sewage conditions.

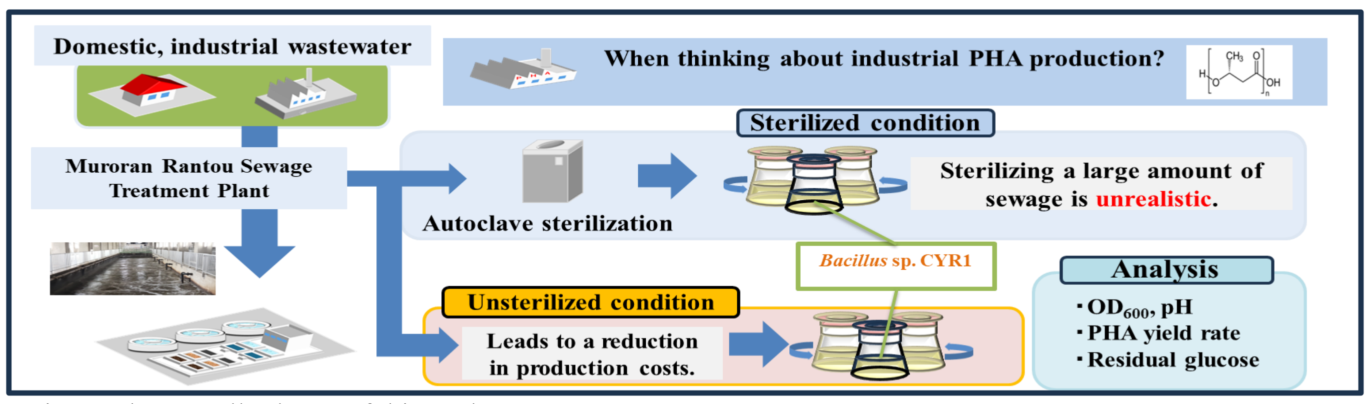
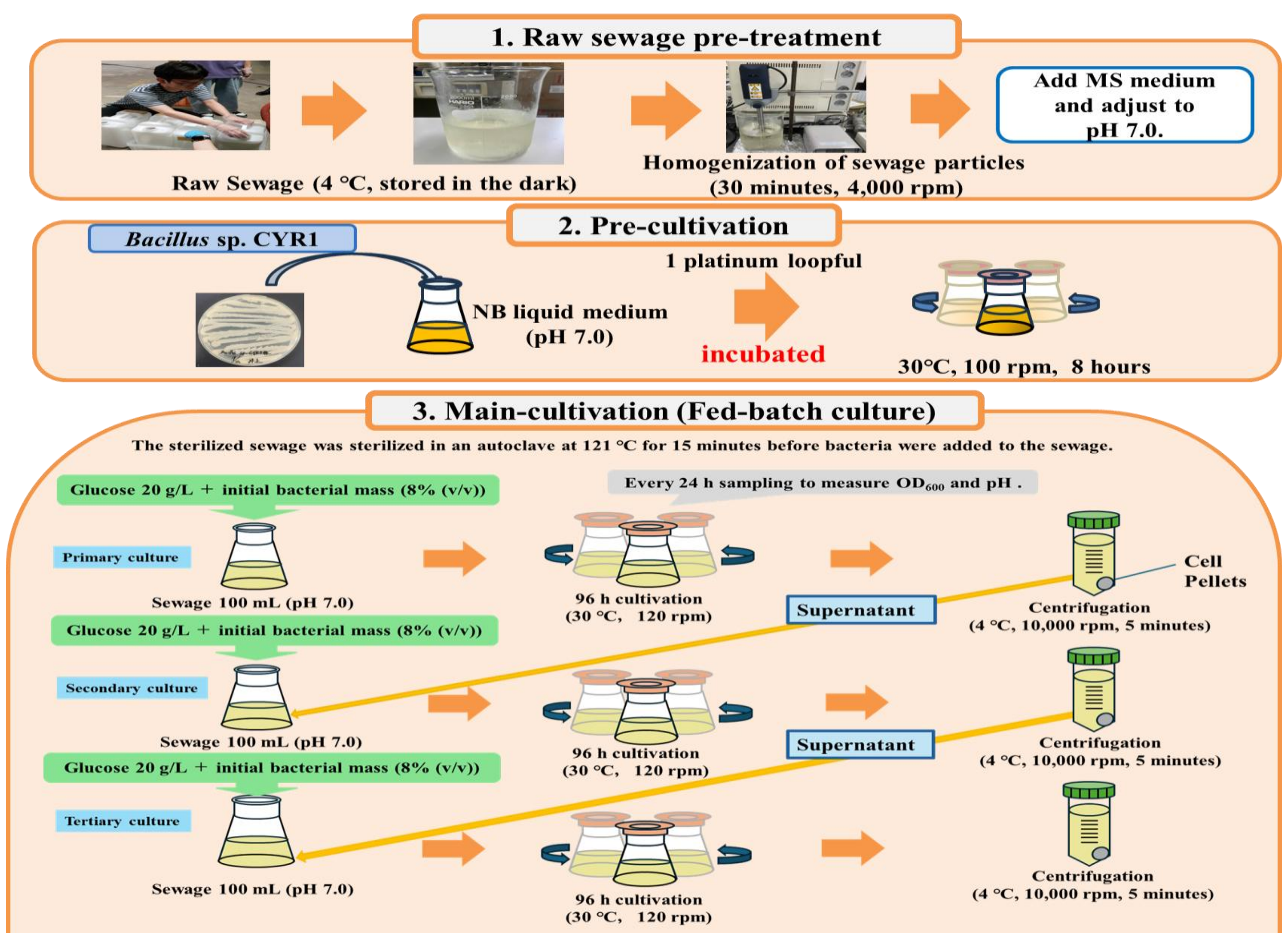


Fig. 1. The overall scheme of this study.

METHOD

Experiments were conducted in 300 mL Erlenmeyer flasks containing 100 mL of sewage water (pH 7.0) supplemented with 20 g/L glucose. *Bacillus* sp. CYR1 was inoculated at 8% (v/v) and aerobically cultured at 30°C with shaking at 120 rpm for 5 days (primary culture). After cultivation, cells were harvested by centrifugation, and the supernatant was reused for secondary culture under identical conditions. This reuse of supernatant continued through tertiary culture until all glucose was completely consumed. To improve PHA yield, we further investigated the effects of increasing the initial inoculum size at the start of each culture and raising the culture temperature to 40°C.



Conditions	Specific experimental methods
Only Raw Sewage Water.	After pre-cultivation, cells were harvested (4 °C, 10,000 rpm, 5 minutes), and the obtained supernatant was added to 100 mL of sewage for culturing at 8% (v/v). (20 g/L glucose was added only at the start of the primary culture.)
20 g/L glucose was added only at the start of the primary culture.	In the secondary and tertiary cultures, glucose was not added.
40 °C condition (Fed-batch culture).	The culture was conducted at 40 °C from the pre-culture to the main culture.
Increasing the initial inoculum size (OD_{600} = 0.5).	After pre-culture, a cell concentration step was performed (4 °C, 10,000 rpm, for 5 minutes), and at the start of each culture, 8% of the pre-culture solution was added to 100 mL of raw sewage water to adjust the OD_{600} to 0.5 (typically around 0.1).

Fig. 2. Overall experimental procedure.

RESULTS & DISCUSSION

Increasing the initial inoculum size (OD_{600} = 0.5).

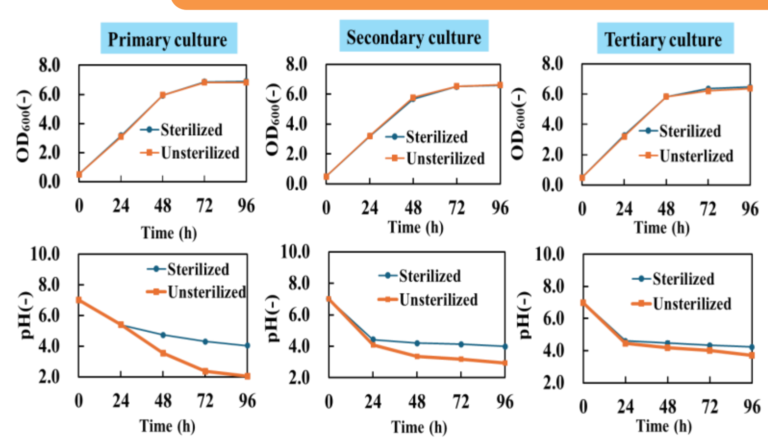


Fig. 3. Comparison of OD_{600} and pH every 24 hours from primary culture to tertiary culture under sterilized and unsterilized conditions (Increasing the initial inoculum size (OD_{600}=0.5)).

Table 1. The values of PHA production yield (%) at each culture stage comparing sterilized and unsterilized conditions (Increasing the initial inoculum size (OD_{600}=0.5)).

Conditions	Stage	Cell dry mass (CDM) (g/L)	PHA production (g/L)	PHA yield rate (%)
Sterilized	Primary	3.26±0.02	2.29±0.12	**70.4±3.77**
	Secondary	3.08±0.16	1.86±0.07	**58.7±2.78**
	Tertiary	2.99±0.02	1.20±0.08	**40.2±2.56**
Unsterilized	Primary	3.12±0.09	1.75±0.07	**56.1±0.88**
	Secondary	3.04±0.11	1.50±0.10	**47.9±4.10**
	tertiary	2.89±0.08	0.92±0.02	**32.1±0.52**

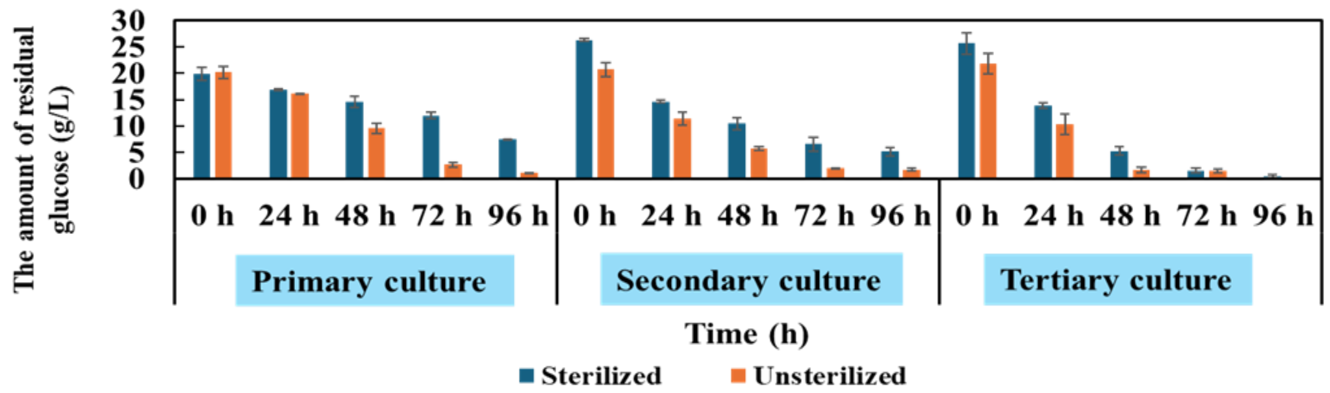


Fig. 4. Comparison of glucose residuals from primary to tertiary culture every 24 hours under sterilized and unsterilized conditions (Increasing the initial inoculum size (OD_{600}=0.5)).

Table 2. The values of PHA production yield (%) at each culture stage comparing sterilized and unsterilized conditions (Culturing under various conditions).

20 g/L glucose was added only at the start of the primary culture.

Conditions	Stage	Cell dry mass (CDM) (g/L)	PHA production (g/L)	PHA yield rate (%)
Sterilized	Primary	1.49±0.15	0.77±0.07	**49.3±5.00**
	Secondary	1.40±0.07	0.74±0.05	**44.4±3.53**
	Tertiary	1.45±0.01	0.77±0.03	**54.0±2.77**
Unsterilized	Primary	1.48±0.10	0.57±0.04	**39.6±3.05**
	Secondary	1.38±0.08	0.54±0.04	**34.4±4.51**
	Tertiary	0.46±0.01	0.08±0.01	**17.2±2.85**

20 g/L glucose was added at the start of all the primary to tertiary cultures (Fed-batch culture).

Conditions	Stage	Cell dry mass (CDM) (g/L)	PHA production (g/L)	PHA yield rate (%)
Sterilized	Primary	1.49±0.03	0.71±0.01	**48.3±0.62**
	Secondary	1.54±0.01	0.78±0.01	**51.4±0.94**
	Tertiary	1.57±0.04	0.89±0.02	**56.6±0.75**
Unsterilized	Primary	1.56±0.03	0.51±0.02	**33.1±0.68**
	Secondary	1.47±0.01	0.61±0.01	**42.4±1.52**
	Tertiary	1.57±0.03	0.76±0.01	**47.9±1.31**

Only Raw Sewage Water condition.

Conditions	Stage	Cell dry mass (CDM) (g/L)	PHA production (g/L)	PHA yield rate (%)
Sterilized	Primary	0.01±0.00	(-)	**(-)**
	Secondary	(-)	(-)	**(-)**
	Tertiary	(-)	(-)	**(-)**
Unsterilized	Primary	1.56±0.08	0.05±0.00	**3.5±0.41**
	Secondary	1.31±0.05	0.03±0.00	**2.8±0.20**
	tertiary	0.44±0.02	0.01±0.00	**0.6±0.04**

40 °C condition (Fed-batch culture).

Conditions	Stage	Cell dry mass (CDM) (g/L)	PHA production (g/L)	PHA yield rate (%)
Sterilized	Primary	1.56±0.06	0.67±1.45	**42.9±1.45**
	Secondary	1.61±0.03	0.71±0.03	**44.3±1.77**
	Tertiary	1.66±0.03	0.80±0.01	**48.4±0.47**
Unsterilized	Primary	1.63±0.01	0.50±0.03	**30.6±1.51**
	Secondary	1.62±0.01	0.60±0.02	**36.7±1.12**
	tertiary	1.62±0.04	0.66±0.02	**40.4±0.51**

Discussion

PHA production under unsterilized sewage conditions was lower than that observed under sterilized sewage conditions, likely because the indigenous bacteria in the raw sewage consumed glucose for their growth, limiting the substrate available for *Bacillus* sp. CYR1 to produce PHA. In addition, increasing the initial inoculum size led to higher PHA production, whereas changing the temperature had no significant effect.

CONCLUSION

Under unsterilized sewage water (raw sewage water) conditions, a maximum PHA production yield of about 56% was achieved using a fed-batch culture (Increasing the initial inoculum size (OD_{600} = 0.5)). The PHA production yield when using raw sewage as a dilution medium was lower than that when using sterilized sewage (maximum 70%). These findings suggest that non-sterilized raw sewage water can serve as a cost-effective and sustainable alternative to sterilized water for industrial-scale PHA production, thereby reducing energy input and improving process feasibility.

FUTURE WORK / REFERENCES

Future research will investigate why the PHA production yield is maximized at this point by measuring the C/N ratio.

References

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[2] Reddy, M. V., Mawatari, Y., Onodera, R., Nakamura, Y., Yajima, Y., & Chang, Y. C. (2019). Bacterial conversion of waste into polyhydroxybutyrate (PHB): A new approach of bio-circular economy for treating waste and energy generation. Bioresource Technology Reports, 7, 100246.