

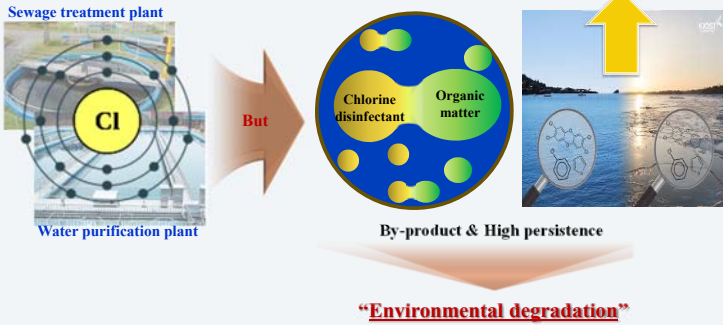
Evaluation of inactivation kinetics and inactivation mechanisms on Gram-negative bacteria using peracetic acid

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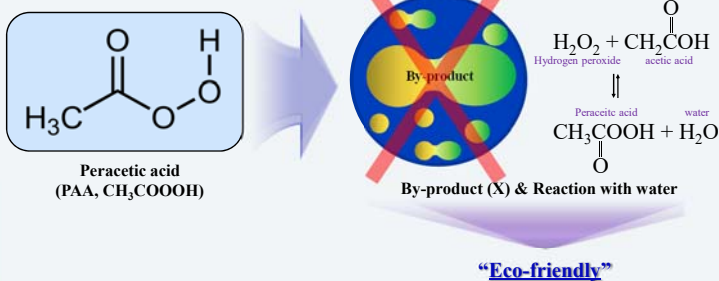
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Introduction

❖ Disadvantages of Chlorine disinfection



❖ Alternative disinfectant

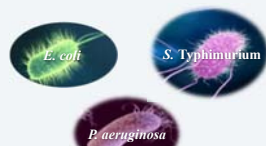
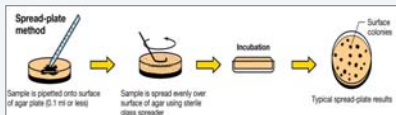


Objective

❖ To evaluate the inactivation kinetics and mechanism of **Gram-negative bacteria** using peracetic acid (PAA).

Experimental Methods

❖ Culture and analysis of target microorganisms



▪ Target: Gram-negative Bacteria

- *E. coli*: Nutrient Broth & Agar (at 37°C for 24 h)

- *S. Typhimurium*: Luria-Bertani Broth & Agar (at 37°C for 24 h)

- *P. aeruginosa*: Luria-Bertani Broth & Agar (at 37°C for 24 h)

❖ Measurement of peracetic acid (PAA)

▪ PROXITANE 15:10 (PAA : ~15%, H₂O₂ : ~10%)

▪ Titration method (Dudley Sully and Williams, 1962)

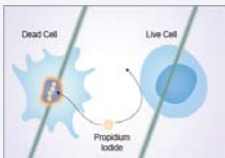
- 1000 μL commercial PAA solution + Deionized water → 250 mL volumetric flask
- 25 mL of aforementioned solution + 10 mL Phosphate buffer + 600 μg of bovine catalase
- After 5 min, 15 mL H₂SO₄ (12 N) and 2.5 g KI are added
- The mixture covered and maintained for 20 min in dark conditions
- 50 mL Deionized water are added, titrated drop by drop with Na₂S₂O₃ (0.1 N) until the disappearance of the color

❖ Inactivation mechanism (~ 1 log inactivation, 90%)

▪ PI staining

▪ API-ZYM assay

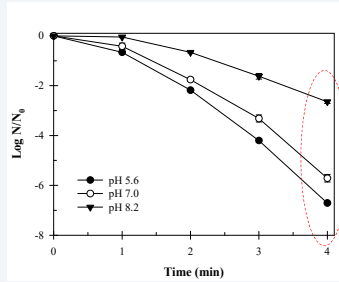
▪ Enzyme degradation (ONPG)



Results and Discussion

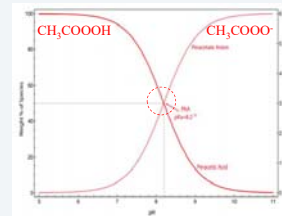
1. Inactivation kinetics of Gram-negative bacteria by PAA

❖ Effect of pH on *E. coli* inactivation



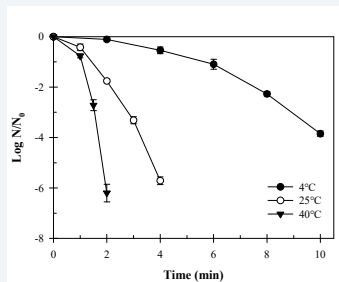
➢ [*E. coli*]₀: 1 * 10⁸ cfu/mL, [PAA]₀: 5 mg/L

More effective in weak acid condition!



➢ Dissociation of PAA with pH change

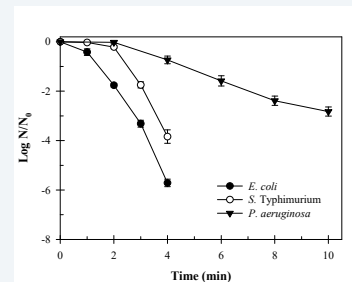
❖ Effect of temperature on *E. coli* inactivation



➢ [*E. coli*]₀: 1 * 10⁸ cfu/mL, [PAA]₀: 5 mg/L, [pH]₀: 7.0

Generally similar to chemical disinfectants (i.e. free chlorine)!

❖ Comparison of Gram-negative bacteria

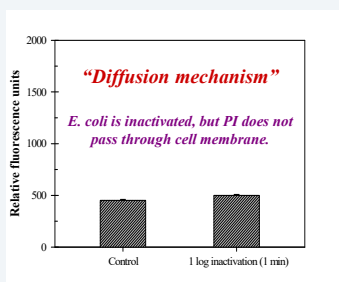


➢ [N]₀: 1 * 10⁸ cfu/mL, [PAA]₀: 5 mg/L, [pH]₀: 7.0

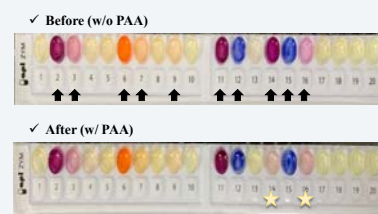
E. coli > *S. Typhimurium* > *P. aeruginosa*

2. Inactivation mechanisms of Gram-negative bacteria (*E. coli*) by PAA

❖ PI staining



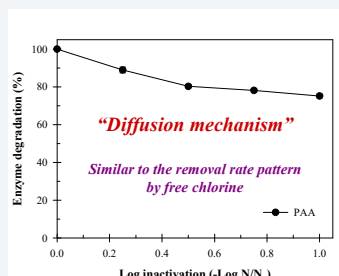
❖ API-ZYM assay



No.	Enzymes	Sample		Standard	
		Before	After	Positive	Negative
1	Control	-	-	Colorless or color of the sample	
2	Alkaline phosphatase	+	+	Violet	
3	Esterase (C4)	+	+	Violet	
4	Esterase Lipase (C8)	-	-	Violet	
5	Lipase (C14)	-	-	Violet	
6	Leucine arylamidase	+	+	Orange	
7	Valine arylamidase	+	+	Orange	
8	Cysteine arylamidase	-	-	Orange	
9	Trypsin	+	+	Orange	
10	α -chymotrypsin	-	-	Orange	
11	Acid phosphatase	+	+	Violet	
12	N-sphol-AS-BI-phosphohydrolase	+	+	Blue	Very pale
13	α -galactosidase	-	-	Violet	Yellow
14	β -glucuronidase	+	-	Violet	
15	β -glucosidase	+	+	Blue	
16	α -glucosidase	+	(-)	Violet	
17	β -glucosidase	-	-	Violet	
18	N-acetyl- β -glucosaminidase	-	-	Brown	
19	α -mannosidase	-	-	Violet	
20	α -fucosidase	-	-	Violet	

+ : positive character, - : negative character, (-) : decreased character

❖ Enzyme degradation (ONPG assay)



Conclusions

▪ PAA (~ 5 mg/L) was effective for inactivating Gram-negative bacteria within 10 min.

- *E. coli* > *S. Typhimurium* > *P. aeruginosa*

▪ Inactivation mechanism: diffusion (i.e. free chlorine)