Degradation of SMX by immobilized bacteria in different types of wastewater

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Background

Remediation using bacteria is cost-effective and prevalent in the breakdown of unwanted products, among which are antibiotics which may be found in many different types of wastewaters. Drawing from previous research[1], *Paenarthrobacter* (P27) and *Nocardioides* (N27) are among some bacteria which may fully degrade the antibiotic sulfamethoxazole (SMX) via initial cleavage of the sulfonamide functional group to form 3A5MI, and 3A5MI degradation respectively, creating an effective combination. To investigate the role of these microbes in SMX degradation, this research is made of four parts, which are **(1)** differences between immobilized degradation bacteria and free degradation bacteria, **(2)** application of immobilized degradation bacteria in different hospital wastewater, **(3)** application of immobilized degradation bacteria in urban sewage, **(4)** application of immobilized degradation bacteria in pig farm wastewater.

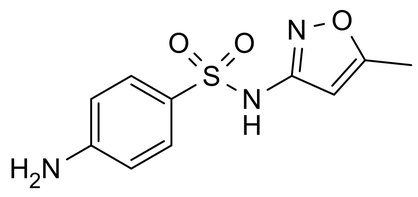


Figure 1: skeletal structure of SMX

Methods

Part 1

Degradation system consists of 25 mL minimal salt medium (MSM, pH = 7) and 50 mg/L sulfamethoxazole (SMX). Inoculation amount is measured by initial concentration of OD_{600} = 0.003, under cultivation conditions of 30^0C and 150 rpm. Immobilized cells are created using a 50:50 solution of sodium alginate and inoculated P27 bacteria and added dropwise into $CaCl_2$ with 1mL syringe. 1mL of immobilized cells are added to each degradation system. All glassware used are autoclaved at 121^0C for 20 minutes. Sample points used are 0, 5, 15, 20, 25, 40 and 50 hours. Two replicates are used for free cell P27 and three replicates are used for immobilized P27. Sample is diluted 25x to be analyzed by HPLC using retention time = 6 minutes and detection λ = 270nm for SMX.

Part 2, Part 3 & Part 4

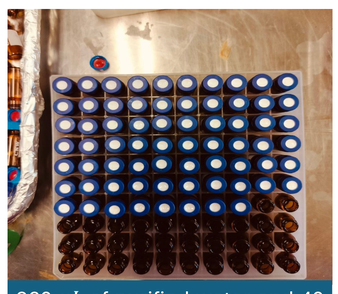
Inoculation amount and conditions, as well as degradation system is similar to part 1, but using 20 mL systems instead. 0.75 mL of immobilized cells are used for the recycled cells in hospital wastewater, pig farm wastewater and urban sewage. Three replicates are used for each wastewater type. Sample points used and analysis conducted are the same as part 1.

MSM and SMX solution are prepared, along with immobilized cells

At designated sample points, 0.3 mL from each system is sampled and stored at 5^0C.

An insightful excursion to the Nanjing Jiang Xin Zhou Sewerage Treatment Plant to learn how sewage water is sampled.

In between sampling, the flasks are stored at 30^0C and 150 rpm in a rotary shaker.



960 μL of purified water and 40 μL of sample is pipetted into each HPLC bottle for analysis.

Results and Discussion

Part 1: differences between immobilized degradation bacteria and free degradation bacteria

Consistent with prior research[2], the degradation activity of P27 in immobilized form is higher than in free cell form. SMX is fully broken down after 40 h by immobilized bacteria as opposed to 50 hours by free cell bacteria.

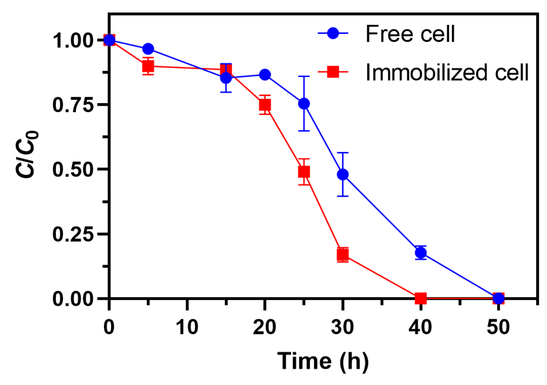


Figure 2: Remaining SMX concentration in salt solution (au) against time after addition of P27 into SMX solution (h)

Immobilised form of microbes also offer advantages such as:
(1) higher biomass concentration, which sped up the catabolism of SMX;
(2) reduced sensitivity to external factors such as pH and temperature; and
(3) easier concentration manipulation[3].

Part 2: application of immobilized degradation bacteria in different hospital wastewater

The inflow and outflow of wastewater from 3 hospitals (E, F and M) are shown by figures 3 and 4. Little to no activity is shown in both inflow and outflow wastewaters for M as well inflow wastewater of F after 50 h, depicting the inability of P27 and N27 in degrading SMX. This may be due to the toxicity of the wastewater environment or intense competition with pre-existing bacteria in those wastewaters, Other microbes can be used to treat SMX for this wastewater. Meanwhile, greater activity of P27 and N27 is observed in the inflow of E, outflow of F and outflow of E.

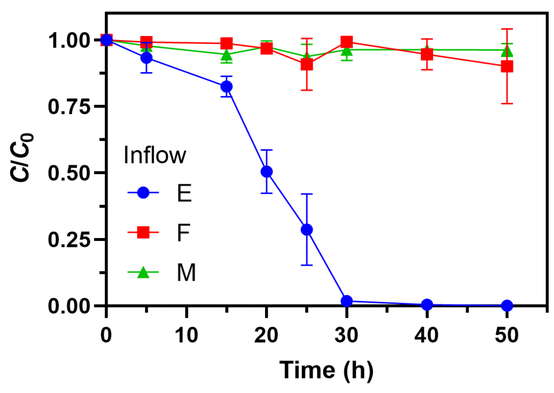


Figure 3: Remaining SMX concentration in influent hospital wastewater (au) against time after addition of P27 and N27.

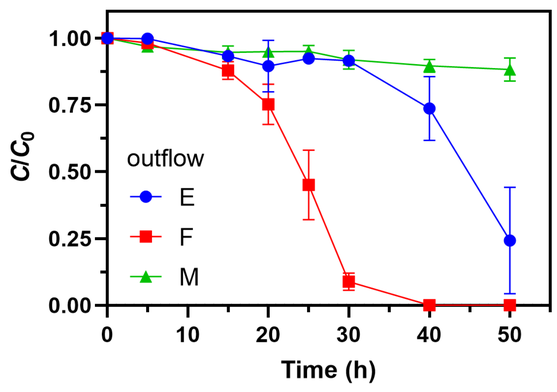


Figure 4: Remaining SMX concentration in effluent hospital wastewater (au) against time after addition of P27 and N27.

Part 3 and 4: application of immobilized degradation bacteria in urban sewage

Results of part 3 and 4 are depicted by figures 5 and 6. At 30 h, immobilized P27 and N27 beads began to rupture in pig farm wastewater, and all fully ruptured at 50 h, suggesting high proliferation of microbes in nutrient-rich pig farm wastewater. Despite the high proliferation rate of microbes, SMX degradation is slower than in urban sewage wastewater where beads did not rupture.

This could be due to:
(a) Higher proliferation rate of N27 than P27, which needs product from degradation of SMX by P27 resulting to high biomass but low degradation;
(b) High abundance of competing organic matter that is used up before SMX
(c) Rupture of beads reduces concentration of bacteria and thus their activity

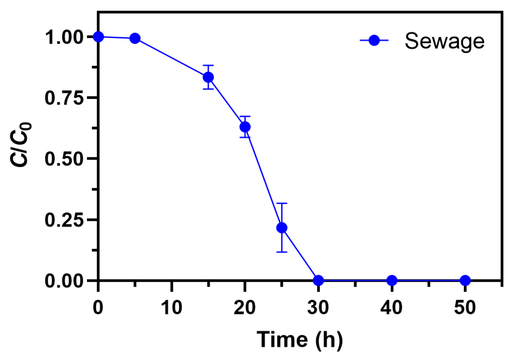


Figure 5: Remaining SMX concentration in urban sewage wastewater (au) against time after addition of P27 and N27.

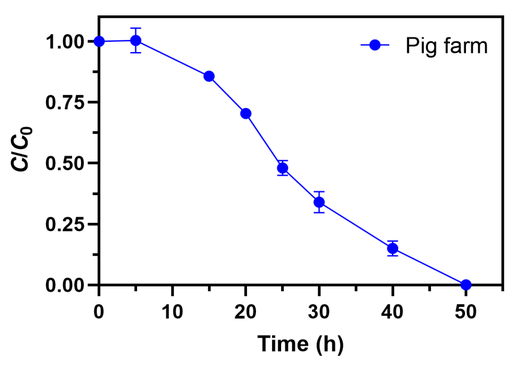


Figure 6: Remaining SMX concentration in pig farm wastewater (au) against time after addition of P27 and N27.

Conclusion

Overall, this experiment demonstrates: (1) the advantages of using immobilized cells as opposed to free cells, (2) the environment medium plays a large part in the overall SMX degradation, (3) comparison of P27 and N27 activity in medium of high and low carbon source.

References

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(2) Wen, S.; Liu, H.; Yang, R.; Wang, L.; Zhu, L.; Wang, J.; Kim, Y. M.; Wang, J. Immobilization of Bacillus Thuringiensis and Applicability in Removal of Sulfamethazine from Soil. *Environmental Pollution* **2023**, *333*, 122080.
(3) Mehrotra, T.; Dev, S.; Banerjee, A.; Chatterjee, A.; Singh, R.; Aggarwal, S. Use of Immobilized Bacteria for Environmental Bioremediation: A Review. *Journal of Environmental Chemical Engineering* **2021**, *9* (5), 105920.