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Comparative study on physico-chemical parameters on biodegradation of phenol by *Pseudomonas aeruginosa* (NCIM 2074) and *Pseudomonas desmolyticum* (NCIM 2028)

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ABSTRACT

Phenols are one of many commonly occurring organic pollutants in the environment. These compounds are stable and even at low concentrations they may be toxic towards living organisms and cause unfavorable chemical changes in waters and soils. Among others, biotechnological methods are applied for their removal. The biodegradation of phenol by *Pseudomonas aeruginosa* (NCIM 2074) and *Pseudomonas desmolyticum* (NCIM 2028), potential biodegradents have been investigated for its degrading potential under different operating conditions. It was found that the degrading potential of *P. aeruginosa* and *P. desmolyticum* was strongly affected by the variations in pH, inoculum size, temperature and carbon (glucose) source. Optimum conditions of the variables for the growth of *P. aeruginosa* and *P. desmolyticum* and for maximum biodegradation of phenol are pH (7, 6), inoculum size (5% v/v, 4% v/v), temperature (32^0C, 32^0C) and carbon (0.5 g/l, 0.5 g/l) respectively. These results are useful to understand the physiological and biochemical properties of *P. aeruginosa* and *P. desmolyticum* before its optimum use in environmental application and these data will assist in choosing the right phenol degrader for a changeable environment.

Key words: Biodegradation, pH, Inoculum size, Temperature, Carbon, Phenol, *Pseudomonas aeruginosa, Pseudomonas desmolyticum.*

INTRODUCTION

Phenol and its derivatives are the basic structural unit in a wide variety of synthetic organic compounds (Annadurai *et al*. 2000). It is an organic, aromatic compound that occurs naturally in the environment (Prpich and Daugulis, 2005), but is more commonly produced artificially from industrial activities such as petroleum processing, plastic manufacturing, resin production, pesticide production, steel manufacturing and the production of paints and varnish (Mahadevaswamy *et al*. 1997; Banyopadhyay *et al*. 1998). This aromatic compound is water soluble and highly mobile (Collins and Daugulis, 1997) and as such wastewaters generated from these industrial activities contain high concentrations of phenolic compounds (Change *et al*. 1998) which eventually may reach down to streams, rivers, lakes, and soil, which represent a serious ecological problem due to their wide spread use and occurrence throughout the environment (Fava *et al*. 1995).

Phenol is a listed priority pollutant by the U.S. Environmental Protection Agency (EPA, 1979) and is considered to be a toxic compound by the Agency for Toxic substances and Disease Registry (ATSDR, 2003). The adverse effects of phenol on health are well documented (Calabrese and Kenyon, 1991) and death among adults has been reported with ingestion of phenol ranging from 1 to 32 gm (Prpich and Daugulis, 2005). The low volatility of

phenol and its affinity for water make oral consumption of contaminated water, the greatest risk to humans (Prpich and Daugulis, 2005).

A variety of techniques have been used for the removal of phenol from industrial effluents and contaminated waters with bioremediation receiving the most attention due to its environmentally friendly, its ability to completely mineralize toxic organic compounds and of low-cost (Kobayashi and Rittman, 1982; Prpich and Daugulis, 2005). Microbial degradation of phenol actively studied and these studies have been shown that phenol can be aerobically degraded by wide variety of fungi and bacteria cultures such as *Candida tropicalis* (Ruiz-ordaz *et al*. 2001; Chang *et al*. 1998; Ruiz-ordaz *et al*. 1998), *Acinetobacter calcoaceticus* (Paller *et al*. 1995), *Alcaligenes eutrophus* (Hughes *et al*. 1984; Leonard and Lindley, 1998), *Pseudomonas putida* (Hill and Robinson, 1975; Nikakhtari and Hill, 2006) and *Burkholderia cepacia* G4 (Folsom *et al*. 1990; Solomon *et al*. 1994). For high strength and low volumes of wastewaters, phenol removal by degradation technique using *Pseudomonas* sp., has been adopted (Annadurai *et al*. 1999).

- The primary factor affecting the substrate decomposition rate in natural systems is pH. Degradation of phenol at various pH, plays a vital role in the growth, degradation and lysis of bacteria. (Annadurai *et al*.1999).
- An effective bacterial inoculum should be able to tolerate high levels of phenol, while maintaining a level of activity to provide efficient mineralization (Shaw et al., 1997). Hence, inoculum size is a primary tool to achieve a good rate of phenol degradation.
- Temperature exerts an important regulatory influence on the rate of metabolism (Ghosh and Swamynathan, 2003).
- Besides, it has been suggested (Roszak and Colwell, 1987; Watanable *et al*. 1998) that other factor, such as the nutrient availability (carbon source), can also affect the bacterial growth.

In order to find a strain able to degrade phenol in a changeable environment, we studied the influence of the pH of the medium, the effect of inoculum size, effect of temperature and carbon source on phenol degradation by phenol degrading organisms - *P. aeruginosa* and *P. desmolyticum* and were compared.

MATERIAL AND METHODS

Chemicals

Phenol (99% pure, chemical grade) 4-amino antipyrine and all other chemicals used were from Merck.

Source of organism

The microorganisms *P. aeruginosa* and *P. desmolyticum* were obtained from culture collection (NCL) Pune, India. The microorganisms were maintained on a medium containing Beef extract: 1.0 g/l, Yeast extract: 2.0 g/l, Peptone: 5.0 g/l, NaCl: 5.0 g/l and Agar: 20 g/l. The pH of the medium was adjusted to 7.0 by adding 1N NaOH. It was stored at 32°C for further use.

Growth determination

To study the extent of degradation, the cells were grown in a Minimal Salts (MS) medium with the following composition: Phenol 0.500 g/l; K_2HPO_4, 1.5 g/l; KH_2PO_4, 0.5 g/l; $(NH_4)_2SO_4$, 0.5 g/l; NaCl, 0.5 g/l; Na_2SO_4, 3.0 g/l; Yeast extract, 2.0 g/l; Ferrous sulfate, 0.002 g/l; $CaCl_2$, 0.002 g/l in conical flasks containing and inoculated with *P. aeruginosa* and *P.desmolyticum*. The experimental studies were carried out in shake flasks with agitation at a rate of 120 rpm, temperature at 32^{0}C. Bacterial growth was determined in terms of cell mass by measuring optical density at a wavelength of 500nm.

Effect of pH of the medium on phenol degradation

Pseudomonas Cells were grown in MS medium with 500 mg/l of phenol at different pH values 5 to 9. This mixture was contained in 250 ml Erlenmeyer flasks. The cultures were placed on a shaker (120 rpm) at 32^{0}C. At different times, growth and phenol degradation were measured.

Effect of inoculum size on phenol degradation

The effect of inoculum size (1 - 10% v/v) on phenol degradation was tested. Cells were grown as shake cultures at 32^{0}C in MS medium supplemented with 500 mg/l phenol at pH 7 in case

of *P. aeruginosa* and pH 6 for *P.desmolyticum* in 250 ml Erlenmeyer flasks. At different times, growth and phenol degradation were measured.

Effect of temperature of the medium on phenol degradation

P. aeruginosa and *P.desmolyticum* were grown in MS medium with 500 mg/l of phenol at different temperatures (30^{0}C, 32^{0}C, 33^{0}C and 34^{0}C) at pH 7, inoculum size 5% v/v and at pH 6, inoculum size 4% v/v respectively. This mixture was contained in 250 ml Erlenmeyer flasks individually. The cultures were placed on a shaker (120 rpm) at the above mentioned temperatures. At different times, growth and phenol degradation were measured.

Effect of carbon (glucose) source on phenol degradation

The effect of carbon (0.0, 0.5, 1, 2, 3 and 4 g/l) on phenol degradation of *P. aeruginosa* and *P.desmolyticum* was tested at pH 7, inoculum size 5% v/v, temperature 32^{0}C, and at pH 6, inoculum size 4% v/v, temperature 32^{0}C respectively. Cells were grown as shake cultures in MS medium supplemented with 500 mg/l phenol in 250 ml Erlenmeyer flask individually. At different times, growth and phenol degradation were measured.

Estimation of phenol

Phenol was determined quantitatively by the Spectrophotometric method (DR/ 4000 V, Hach) using 4-amino antipyrine as the color reagent (λ_{max}: 500nm) according to standard methods of analysis (APHA, 1989).

Growth determination

Bacterial growth was determined in terms of cell mass by measuring optical density at a wavelength of 500nm.

RESULTS AND DISCUSSION

Biological treatment using *P. aeruginosa* and *P.desmolyticum* was the effective method for removal of phenol. It is also a time saving method compared to other conventional methods.

Effect of pH of the medium on phenol degradation

pH values from 5 to 9 were investigated. Phenol was degraded rapidly at pH 7 by *P. aeruginosa* and at pH 6 by *P.desmolyticum*. In control cultures no aromatic ring cleavage occurred. At these pH values, phenol degradation was high compared to other pH values. The phenol concentration started decreasing after 24 h inoculation for both the microorganisms but the rate of degradation of phenol was high and faster by *P. aeruginosa* compared to *P.desmolyticum* as shown in (Fig. 1).

The internal environment of all living cells is believed to be approximately neutral. Most organisms cannot tolerate pH levels below 4.0 or above 9.0. At low (4.0) or high (9.0) pH values, acid or bases can penetrate into cells more easily because they tend to exist in undissociated forms under these conditions and electrostatic force cannot prevent them from entering the cells. The permeated substances can upset the internal pH balance since the bacterial activity decreases as the pH deviates from neutral conditions.

Effect of inoculum size on phenol degradation

Phenol was degraded by *P. aeruginosa* and by *P.desmolyticum* during all the inoculum sizes (1-10% v/v) tested at pH 7 and at pH 6 respectively. At 5% v/v, *P. aeruginosa* and at 4% v/v, *P.desmolyticum* the phenol concentration began to decrease rapidly and complete degradation has taken place at 60 h for *P. aeruginosa* and at 70 h by *P.desmolyticum*. Cultures inoculated with 5 % v/v and 4% v/v inoculum sizes for *P. aeruginosa* and by *P.desmolyticum* showed the highest rate of phenol degradation as shown in Fig.-2, while the cultures inoculated with the other inoculum sizes showed a decrease in phenol consumption.

Effect of temperature of the medium on phenol degradation

Temperature exerts an important regulatory influence on the rate of metabolism. However, some work has been done on the microbiological activity of the organisms present in the water treatment plants operating at lower temperatures. But conventional biological waste treatment processes can operate at low temperature provided sufficient time is allowed for these organisms to degrade in organic wastes. Microbiological degradation of phenol in industrial

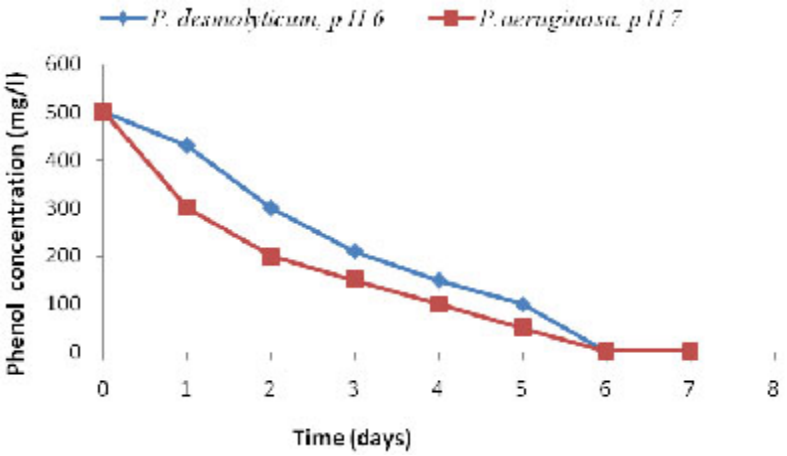

Fig. 1: Effect of pH on phenol degradation by *P.aeruginosa* and *P. desmolyticum*

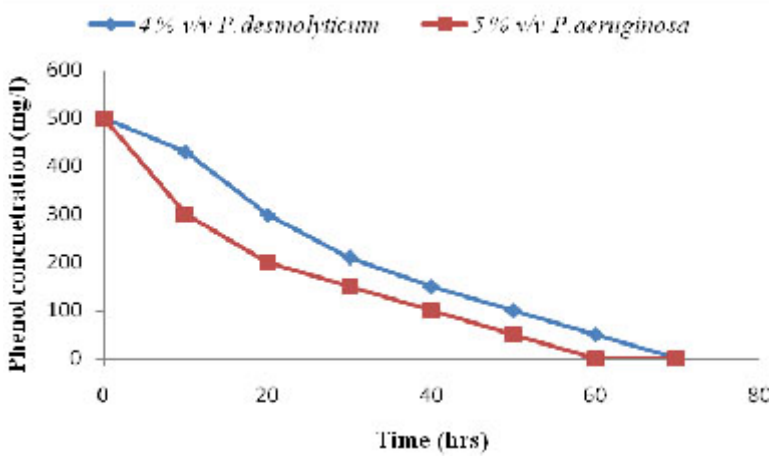

Fig. 2: Effect of inoculum size on phenol degradation by *P. aeruginosa* and *P. desmolyticum*

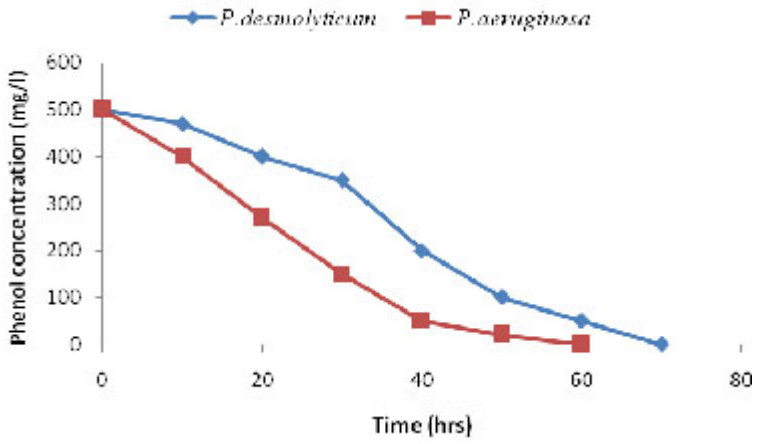

Fig. 3: Effect of Temperature on phenol degradation by by *P.aeruginosa* and *P.desmolyticum*

wastewater is affected by temperature in an unexpected manner. The efficiency of treatment by microbiological activity on phenol and other contaminants were significantly good.

Temperature values (30°C, 32°C, 33°C and 34°C) were investigated for *P. aeruginosa* (pH 7, 5% v/v) and *P. desmolyticum* (pH 6, 4% v/v). Both the microorganisms degraded phenol rapidly at temperature 32°C. At this temperature, the phenol concentration began to decrease rapidly after 24 h inoculation and degraded completely around 60 h by *P. aeruginosa*. Phenol was degraded rapidly by *P. desmolyticum* at 32^{0}C after 40hrs and complete degradation was found at around 70 h as shown in Fig.-3. Hence, *P.aeruginosa* had better degradation rate than *P. desmolyticum.*

Effect of carbon (glucose) source on phenol degradation

The presence of glucose in the culture medium increased the tolerance of the organisms to high phenol concentrations by providing a good source readily metabolisable carbon to support cell growth. Hence, it was concluded that glucose on MS medium supported phenol degradation.

Phenol degradation by *P. aeruginosa* and *P. desmolyticum* at different concentrations of glucose (0.0, 0.5, 1.0, 2.0, 3.0, 4.0 g/l) was tested.

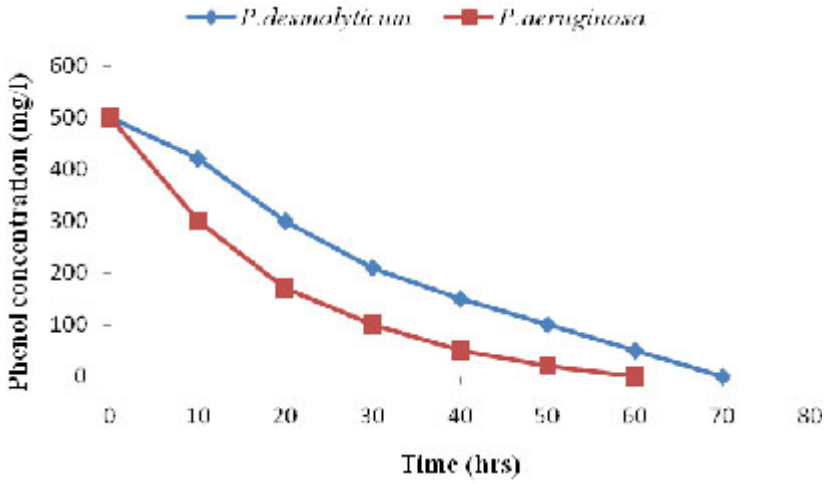

Fig. 4: Effect of glucose on phenol degradation by by *P.aeruginosa* and *P.desmolyticum*

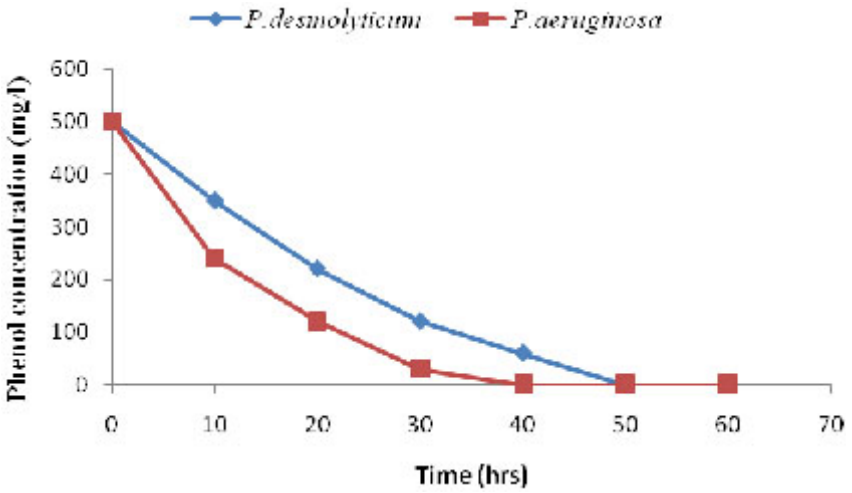

Fig. 5: Experimental values of phenol concentration during fermentations by *P.aeruginosa* (pH 6, 4% v/v, 32°C, 0.5 g/l) and *P.desmolyticum* (pH 6, 4% v/v, 32°C, 0.5 g/l)

Cultures inoculated with 0.5 g/l glucose for *P.aeruginosa* (pH 7, 5% v/v, 32^{0}C) and *P. desmolyticum* (pH 6, 4% v/v, 32^{0}C) showed the highest rate of phenol degradation as shown in Fig.-4, while the cultures inoculated with the other concentrations showed a decrease in phenol consumption.

CONCLUSION

These results shows *P. aeruginosa* is able to tolerate higher levels of phenol when supplemented with glucose as additional source compared to *P. desmolyticum.* Time is the limiting factor as the biodegradation of recalcitrant compounds can range from hours to years.

In Fig. 5 the variation of phenol concentration at their optimum conditions throughout both fermentations are shown. As can be seen, the removing rate of phenol by *P.aeruginosa* is slightly higher than that of *P.desmolyticum.*

REFERENCES

1. Annadurai, G., Balan, S.M. and Murugesan, T. Design of experiments in the biodegradation of phenol using immobilized *Pseudomonas pictorium* (NCIM-2077) on activated carbon. *Bioproc. Eng*. **22**: 101-107 (2000).
2. Prpich, G.P. and Daugulis, A.J., Enhanced biodegradation of phenol by a microbial consortium in a solid-liquid two-phase partitioning bioreactor. *Biodegradation.* **16**: 329-339 (2005).
3. Mahadevaswamy, M., Mall, I.D., Prasad, B. and Mishra, I.M., Removal of phenol by adsorption on coal fly ash and activated carbon. *Pollut. Res.* **16**(3): 170-175 (1997).
4. Bandyopadhyay, K., Das, D. and Maiti, B.R., Kinetics of phenol degradation using *Pseudomonas putida* MTCC 1194. *Bioproc. Eng*. **18**: 373-377 (1998).
5. Collins, L.D. and Daugulis, A.J. Biodegradation of phenol at high initial concentration in two-phase partitioning batch and fed-batch bioreactors. *Biotechnol. Bioeng*. **55**: 155-162 (1997).
6. Change, Y.H., Li, C.T., Chang, M.C. and. Shieh, W.K. Batch phenol degradation by *Candida tropicalis* and its fusant. *Biotechnol. Bioeng.* **60**: 391-395 (1998).
7. Fava, F., Armenante, P.M., Kafkewitz, D. and Marchetti, L, Influence of organic and inorganic growth supplements on the aerobic biodegradation of chlorobenzoic acid. *Appl. Microbial. Biotechnol*. **43**: 171-177 (1995).
8. Calabrese, E.J. and Kenyon, E.M. Air toxics and Risk Assessment. Lewis publishers, Chelsea. MI (1991).
9. Kobayashi, H. and Rittman, B.E., Microbial removal of hazardous organic compounds. *Environ. Sci. Technol*. **16**: 170-183 (1982).
10. Ruiz-ordaz, N., Ruiz-Lagunez, J.C., Castanou-Gonzalez, J.H., Hernandez-Manzano, E., Cristiani-Urbina, E. and Galindez-Mayer, J. Phenol biodegradation using a repeated batch culture of *Candida tropicalis* in a multistage bubble column. *Revista Latinoamericana de Microbiogia*. **43**: 19-25 (2001).
11. Ruiz-ordaz, N., Ruiz-Lagunez, J.C., Castanou-Gonzalez, J.H., Hernandez-Manzano, E., Cristiani-Urbina, E. and Galindez-Mayer, J. Growth kinetic model that describes the inhibitory and lytic effects of phenol on *Candida tropicalis* yeast. *Biotechnol. Prog*. **14**: 966-969 (1998).
12. Paller, G., Hommel, R.K and Kleber, H.P., Phenol degradation by *Acinetobacter calcoaceticus* NCIB 8250. *J. Basic Microbial.* **35**: 325-335 (1995).
13. Hughes, E.J., Bayly, R.C. and Skurray, R.A. Evidence for isofunctional enzymes in the degradation of phenol , m- and p- toluate, and p- cresol via catechol metacleavage pathways in *Alcaligenes eutrophus*. *J. Bacteriol*. **158**: 79-83 (1984).
14. Leonard, D. and Lindley, N.D. Carbon and energy flux constraints in continuous cultures

of *Alcaligenes eutrophus* grown on phenol. *Microbiology.* **144**: 241-248 (1998).

15. Hill, G.A. and Robinson, C.W., Substrate inhibition kinetics: Phenol degradation by *Pseudomonas putida*. *Biotechnol. Bioeng.* **17**: 599-615 (1975).
16. Nikakhtari, H. and Hill , G.A. Continuation bioremediation of phenol- polluted air in an external loop airlift bioreactor with a packed bed. *J. Chem. Tech. Biotechnol.* **81**(6): 1029-1038 (2006).
17. Folsom, B.R., Chapman, P. J. and Pritchard, P.H., Phenol and trichloroethylene degradation by *Pseudomonas cepacia* G4: Kinetics and interactions between substrates. *Appl. Environ. Microbial.* **57**: 1279-1285 (1990).
18. Solomon, B.O., Posten, C., Harder, M.P.F., Hecht, V. and Deckwer, W-D., Energetics of *Pseudomonas cepacia* growth in a chemostat phenol limitation. *J. Chem. Technol. Biotechnol*. **60**: 275-282 (1994).
19. Annadurai, G., Mathalai Balan, S. and Murugesan, T. Box- Behnken design in the development of optimized complex medium for phenol degradation using *Pseudomonas putida* (NCIM 2174). *Bioproc. Eng.* **21**: 415-421 (1999).
20. Shaw, K.W., Lee, H. and Trevors, J. Effect of initial cell density, substrate concentration and temperature on penta chloro phenol degradation by *Pseudomonas* sp., UG-30. *J. Chem. Technol. Biotechnol.* **69**: 107-113 (1997).
21. Ghosh, S. and Swaminathan, T. Optimization of process variables for the extractive fermentation of 2,3 – butanedion by *Klebsiella oxytoca* in aqueous two- phase system using response surface methodology. *Chem. Biochem. Eng.* Q **17**(4): 319-325 (2003).
22. Roszak, D.B. and Colwell, R.R. Survival strategy of bacteria in the natural environment. *Microbiol. Rev.* **51**: 207-214 (1987).
23. Watanabe, K., Yamamoto, S., Hino, S. and Harayama, S. Population dynamics if phenol-degrading bacteria in activated sludge determination by *gyrB*-targeted quantitative PCR. *Appl. Environ. Microbiol.* **64**: 1203-1209 (1998).
24. American Public Health Association (APHA), American Water Works Association, Water Pollution Control Federation. Standard methods for the examination of water and wastewater. 17th edition. Washington, D.C. American Public Health Association. 9-55-9-62 (1989).