

Influence of Kinetic and Metabolic Selection on 17 α -ethinylestradiol Biodegradation in
Activated Sludge Wastewater Treatment Systems

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Abstract

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The potent endocrine-disrupting estrogen hormone, 17α -ethinylestradiol (EE2), is primarily removed via biodegradation in municipal wastewater treatment plant (WWTP) activated sludge (AS) processes; however, reported EE2 removal efficiencies in AS WWTPs vary widely. A hypothesis of this research was that EE2 biodegradation kinetics vary as a function of AS process and reactor designs, which select for different microbial population compositions. Bench-scale AS reactors treating municipal wastewater and estrogens at ng/L concentrations were operated to simulate kinetic population selection with high initial food-to-biomass ratio feeding conditions (high-F/M_f) or low substrate growth conditions (low-F/M_f), as well as metabolic selection with substrate uptake and growth under aerobic, anaerobic, and anoxic conditions. The latter two metabolic selectors resulted in enhanced biological phosphorus removal and biological nitrogen removal, respectively. A pseudo first-order biodegradation model was used to examine the effects of metabolic and kinetic selective pressures on EE2 biodegradation kinetics. Aerobic low-F/M_f reactors experienced pseudo first-order EE2

biodegradation rate coefficients (k_b) that were 1.4 to 2.2 times greater than high-F/M_f aerobic selectors operated in parallel, suggesting that kinetic selection influences EE2 biodegradation activity in AS systems. No significant difference was observed in the EE2 k_b of high-F/M_f metabolic bioselectors (aerobic-only, anoxic/aerobic, and anaerobic/aerobic). However, metabolic selection reduced the EE2 k_b of a low-F/M_f anoxic/aerobic reactor by 40% relative to a low-F/M_f aerobic reactor, demonstrating that the redox state of growth conditions may affect microbial EE2 biodegradation kinetics in AS. The results of this study suggest that operating conditions in which microbial growth occurs aerobically at low substrate concentrations improve EE2 biodegradation kinetics in AS systems, possibly due to the growth of K-strategist heterotrophs capable of more efficient EE2 biodegradation at low ng/L concentrations.

Supplementary files to this dissertation include Appendix B, which is a Microsoft Excel file that provides supporting data to the presented research results.

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INTRODUCTION

Natural and synthetic estrogens of human origin are endocrine disrupting compounds (EDCs) capable of harming the sexual and reproductive processes of aquatic wildlife and fish.¹⁻³ Estrogens of particular environmental concern include the natural estrogens estrone (E1) and 17 β -estradiol (E2), and the synthetic estrogen 17 α -ethinylestradiol (EE2). EE2 exerts an EDC-potency that may be up to 1.25 times that of E2 and over 15 times that of E1.⁴ Aquatic exposure to EE2 can result in reproductive and sexual damage to fish species at concentrations below 1 ng/L.^{2,5} Municipal wastewater treatment plant (WWTP) effluents are major contributors of estrogens to aqueous environments^{3,6}, with EE2 detected in WWTP effluents at concentrations up to 42 ng/L after activated sludge (AS) treatment⁸.

Biodegradation is the major removal mechanism for estrogens in AS treatment⁷, with considerably lower biodegradation rates observed for EE2 than for E1 and E2⁸. Heterotrophic bacteria are considered to be major contributors to EE2 biodegradation^{9,10}, and have been shown to degrade EE2 in pure-culture¹¹⁻¹³. Yet, heterotrophic EE2-degrading bacteria must grow on other carbon substrates in AS, as the ng/L EE2 concentrations in municipal wastewater could not alone support sufficient biomass growth to account for the observed levels of estrogen removal in AS WWTPs. Presently no method is available to quantify the fraction of EE2-degrading heterotrophic bacteria in AS mixed liquor. Yet, differences in the concentration of EE2-degrading bacteria may be one factor contributing to the unexplained variation in reported EE2 removal efficiencies at municipal AS WWTPs, which range from 0 to over 98 percent¹⁴⁻²⁰.

The heterotrophic bacterial composition in AS treatment can be affected by the type of growth substrate, operating conditions, and reactor configuration.²¹ Improved removal of EDCs in AS treatment has been suggested to result from the development of a more a physiologically diverse

microbial consortium with operation at longer AS solids retention times (SRTs).^{16,22} However, in addition to changing the SRT, microbial composition in AS treatment is commonly altered by the use of reactor configurations and/or redox conditions that provide kinetic or metabolic selective pressures to enrich for bacterial communities with improved sludge settling characteristics and/or biological nutrient removal.^{21,23} Kinetic selectors have been used to favor floc-forming bacteria over filamentous bacteria by using high initial contact food-to-biomass ratio (F/M_f) feeding conditions, such as are achieved with compartmentalized AS systems and rapid fill/react times in sequencing batch reactors (SBRs).²³ Kinetic population selection for filamentous bacteria control favors microorganisms termed *r-strategists*, which have greater maximum specific growth rates and are capable of rapid substrate uptake at higher substrate loadings, rather than filamentous *K-strategists*, which have lower Michaelis-Menten half-saturation coefficient values.²⁴ Alternatively, metabolic population selection relies upon anoxic or anaerobic redox conditions to favor substrate uptake by heterotrophic bacteria capable of nitrate/nitrite reduction or high intracellular polyphosphate storage, respectively.²³ Common metabolic selector designs include sequential anoxic and aerobic reactors for nitrogen removal, as well as sequential anaerobic, anoxic, and aerobic reactors for enhanced biological phosphorous removal (EBPR).²³ Metabolic selection can be augmented by kinetic selection when anoxic or anaerobic redox conditions are employed at high initial reactor substrate concentrations to further prevent filamentous growth.²³

The effect of AS population selection mechanisms on the activity of EE2-degrading microorganisms is poorly understood. A comparison of estrogen removal at two full-scale WWTPs with and without an anaerobic selector revealed differences in EE2 biomass biodegradation activities¹⁸, and emphasized the need to further evaluate the influence of

metabolic selector AS designs on EE2 biodegradation kinetics. Moreover, AS systems with longer SRTs and lower F/M ratios are thought to have improved estrogen removal efficiencies due to the growth of slow-growing specialized microorganisms.^{16,22} Yet, to the best of our knowledge, no study has compared all types of metabolic selectors with the same influent wastewater source and SRT in order to determine if distinct heterotrophic biological nutrient removal (BNR) populations express different EE2 biodegradation kinetics; nor has a study been conducted to compare the EE2 biodegradation kinetics of r- and K-strategist heterotrophic populations by operating AS systems with identical SRTs, but with substrate uptake occurring under high or low concentrations. In this study, we hypothesized that heterotrophic EE2 degradation is not ubiquitous, and thus different EE2 biodegradation rate coefficients will be observed in different AS reactor configurations that use kinetic and metabolic growth pressures to select for distinct heterotrophic populations.

The objective of this study was to investigate the effect of metabolic and kinetic biological selection processes on EE2 biodegradation kinetics during AS treatment of municipal primary effluent wastewater with estrogens at ng/L concentrations. Pseudo first-order EE2 biodegradation rate coefficients normalized to mixed liquor volatile suspended solids (MLVSS) were used to characterize the EE2 biodegradation kinetics for different selector operating conditions in AS SBRs. Metabolic population selection was examined by operating SBRs with aerobic, anoxic, or anaerobic redox conditions during the fill and react period. Kinetic selection conditions were promoted by feeding SBRs over a short duration with a high initial contact F/M_f for substrate uptake at a high concentration, or conversely feeding over the entire SBR react period with a low F/M_f for substrate uptake at a low concentration.

MATERIALS AND METHODS

Activated Sludge Reactor Configuration and Operation

Three sets of AS SBRs were operated in this study to examine the effects of metabolic and kinetic population selection mechanisms on EE2 biodegradation kinetics. An overview of the experimental design for the three SBR Phases is given in Table 1. Phase I examined the effect of the three types of metabolic selectors that employed feeding conditions expected to favor r-strategists. Phase II examined the impact of kinetic growth conditions expected to favor r- or K-strategists under aerobic conditions. Phase III examined the effect of growth environments expected to favor K-strategists under aerobic and anoxic metabolic conditions, and also compared kinetic growth conditions expected to favor r- or K-strategists under aerobic conditions. Activated sludge seed from local WWTPs was added to the SBRs at the start of each phase; City of Snoqualmie oxidation ditch for Phase I, City of Port Orchard conventional AS and Phase I aerobic AS mixed liquor for Phase II, and King County South WWTP (Renton) aerobic AS for Phase III. The parallel reactors in each operating Phase received equal amounts of COD each day, and their initial feeding food-to-biomass ratio (F/M_f) loading was equal to the COD feed rate during the feeding period (g COD/day) divided by the reactor MLVSS mass. Higher F/M_f loading conditions were implemented using shorter feed durations in some AS reactors to promote substrate uptake at initially high concentrations and select for r-strategist organisms; such operation will from hereon be referred to as “high- F/M_f ”. To select for K-strategists with operation at a low substrate, the feed was added semi-continuously throughout the react/fill period to simulate a low-loaded single-tank complete mix AS reactor; this operation will from hereon be referred to as “low- F/M_f ” to signify the feeding conditions used to promote growth at low substrate.

Table 1. SBR Operating Conditions for the Three Experimental Phases Used to Investigate the Effects of Population Selection Processes on EE2 Biodegradation Kinetics.

Phase	Feeding Strategy	Kinetic Selection Type	Fill Time per Cycle (min)	Metabolic Selection in Fill/React Period
I Metabolic selection at high-F/M _f	High-F/M _f	r-	15	Aerobic
	High-F/M _f	r-	15	Anoxic
	High-F/M _f	r-	15	Anaerobic
II Kinetic selection	High-F/M _f	r-	15	Aerobic
	Low-F/M _f	K-	300	Aerobic ^a
	High- F/M _f ^b	r-	15	Aerobic
III Kinetic selection and metabolic selection at low-F/M _f	High-F/M _f	r-	5	Aerobic
	Low-F/M _f	K-	300	Aerobic ^a
	Low-F/M _f	K-	300	Anoxic/Aerobic ^a

Key: SBR=sequencing batch reactor; F/M_f=initial feeding food-to-biomass ratio; r-=r-strategist type; K-=K-strategist type; rbCOD=readily biodegradable chemical oxygen demand

^a Feeding occurred throughout react period

^b Lower influent rbCOD

The AS SBRs operated in this study treated primary effluent from King County West Point WWTP (Phases I and II) and King County South WWTP (Phase III) augmented with additional substrate and the estrogens E1, E2 and EE2. Equal quantities of acetate were added to the feed of the SBRs during each operating Phase at approximately 120 mg/L for Phases I and II and 150 mg/L for Phase III. Sodium nitrate was added to the feed of the anoxic/aerobic selectors to increase the fraction of denitrifiers in the total population. Phosphate as mono and dibasic potassium phosphate was added to the feed in Phases I and II.

The Phase I and II SBRs were operated at the West Point WWTP in Seattle, WA at ambient temperatures, with daily replenishment of their primary effluent feed container. The Phase III SBRs were operated in a 20°C walk-in environmental chamber at the University of Washington

Environmental Engineering Laboratory, and the King County South WWTP primary effluent wastewater feed was stored at 4°C after its delivery every 7 days. Each SBR consisted of a 4-L Pyrex glass Erlenmeyer flask. The total fill liquid volume was 4.0 L for Phases I and II and 3.8 L for Phase III. All SBRs had a total cycle time of 6.0 hours, and the feed volume for each cycle was one-quarter of the total liquid volume for Phases I and II and one-fifth for Phase III. Magnetic stir bars mixed the AS SBRs throughout the fill and react periods. A quiescent settling time of 1.0-hour occurred before supernatant was decanted throughout the final 8.0 min of the cycle with peristaltic pumps. Aeration was provided with an aquarium pump, and mixed-liquor nitrogen sparging was conducted in the anoxic and anaerobic metabolic selector periods to eliminate oxygen surface transfer. A ChronTrol-XT timer controlled the feed, sludge wasting, and effluent withdrawal pumps, as well as the reactor mixers, aeration pumps, and nitrogen solenoid valves.

Mixed liquor was wasted daily to maintain the target total SRT based on the prior day's effluent and mixed liquor suspended solids concentrations. The walls of each reactor were scraped daily to minimize biofilm growth. Samples for mixed liquor suspended solids (MLSS), MLVSS, and pH were collected manually during the aerobic cycle of each reactor at a frequency of five times per week.

Details of the AS SBR operational parameters, and average influent and effluent data are summarized in Table 2. Operational details unique to each operating Phase are summarized below.

Phase I Operation: The mixed-liquor of the anoxic/aerobic and anaerobic/aerobic SBRs was purged with nitrogen gas at 300 mL/min during the initial 1.5 hours of each cycle. This purging began five-minutes before feeding in order to strip any remaining saturated oxygen and eliminate

surface transfer. Half of the synthetic acetate solution (5mL) was delivered along with primary effluent to each reactor at the start of the cycle, as was 10 mL of nitrate solution to the anoxic/aerobic reactor. After 45-minutes of operation, the remaining 5 mL of the synthetic acetate solution was added to each reactor. Staging the feed in this manner ensured that readily degradable chemical oxygen demand (rbCOD) was available to phosphorus accumulating organisms (PAOs) in the anaerobic/aerobic selector once the nitrate was fully reduced. The metabolic selectors were aerated for 3.5 hours after their anoxic and anaerobic fill/react periods, while the aerobic selector was aerated during the first 3.5 hours of the cycle. The aerobic SBR remained idle for 1.5 hours after settling and decanting to account for the anoxic and anaerobic periods in the metabolic selector SBRs. Influent concentrations of E1, E2, and EE2 to the Phase I SBRs averaged 213 ± 41 ng/L, 176 ± 28 ng/L, and 164 ± 25 ng/L, respectively ($n=21$).

The effluent supernatant decanted after the settling period in each reactor was stored at 4°C prior to sampling. Reactor effluent samples were analyzed five days per week for total suspended solids (TSS) and volatile suspended solids (VSS). Key nutrient parameters, including $\text{NH}_3\text{-N}$, $\text{NO}_3\text{-N}$, sCOD, and $\text{PO}_4\text{-P}$ were also analyzed in the effluent samples three times weekly. Composite averages of the primary effluent characteristics were obtained by sampling before and after its daily replacement. Composite primary effluent nutrient samples were measured three times weekly, and composite primary effluent solids were measured five times weekly.

Phase II Operation: One of the high- F/M_f aerobic selectors and the low- F/M_f reactor received the same amount of supplemental acetate at approximately 120 mg/L, while the other high- F/M_f aerobic selector did not receive any acetate to reduce its influent rbCOD. The Phase II SBRs were aerated for the entire 5-hour fill/react period before settling. The two aerobic high- F/M_f SBRs were fed primary effluent and synthetic nutrients over the first 15-minutes of the 5-hour

fill/react period. Complete-mix conditions were simulated in the aerobic low-F/M_f reactor by delivering wastewater feed throughout the 5-hour fill/react period with 15-second feedings at a frequency of 5-minutes using calibrated peristaltic pumps. Influent concentrations of E1 and EE2 to the Phase II SBRs averaged 192 ± 35 ng/L and 118 ± 25 ng/L ($n=12$), respectively, while E2 was somewhat reduced in the Phase II feed at 70 ± 54 ng/L ($n=12$) possibly due to transformation during storage. The Phase II reactor effluents were stored and sampled as described above for Phase I operation.

Phase III Operation: The King County South WWTP primary effluent feed was augmented with 30 mg-TSS/L as primary sludge collected from the King County West Point WWTP in Seattle, WA to increase the influent inert solids and aid settling in the aerobic low-F/M_f reactor. Sodium bicarbonate (NaHCO₃) was added to the primary effluent wastewater feed to maintain an alkalinity concentration of 100 mg/L as CaCO₃. The high-F/M_f aerobic selector was fed primary effluent and synthetic nutrients over the first 5-minutes of the 5-hour fill/react period. Complete-mix conditions were simulated in the two low-F/M_f reactors by delivering wastewater feed throughout their 5-hour fill/react periods with 8-second feedings at a frequency of 3-minutes using calibrated peristaltic pumps and a timer control. The high-F/M_f aerobic selector and aerobic low-F/M_f reactor were aerated throughout the 5-hour fill/react period before settling. The anoxic/aerobic low-F/M_f reactor was purged with nitrogen gas at 300 mL/min for the first two-hours (40%) of the fill/react period, and was subsequently aerated for the remaining 3-hours (60%) of the fill/react period. Influent concentrations of E1, E2, and EE2 in Phase III averaged 448 ± 100 ng/L, 348 ± 75 ng/L, and 301 ± 17 ng/L, respectively ($n=23$).

Key nutrient parameters, including NH₃-N, NO₃-N, sCOD, and PO₄-P were analyzed in mixed liquor samples collected at the end of the aerobic period three days weekly. The combined

primary effluent and supplemented nutrient wastewater feed was sampled for TSS, VSS, NH₃-N, NO₃-N, TCOD, sCOD, and PO₄-P at least two days weekly.

EE2 Batch Degradation Tests

All reactors were operated for a minimum of three SRTs before conducting EE2 batch degradation tests. EE2 degradation tests for the Phase I reactors were performed as in-situ batch tests, in which estrogen concentrations were monitored throughout the aeration period after feeding. Only the estrogens present in the influent wastewater matrix were added to the reactors during in-situ batch degradation tests in Phase I. Because of the semi-continuous feeding strategy for the low-F/M_f operation employed in Phases II and III, EE2 degradation tests for those reactors were conducted as ex-situ batch tests. In the ex-situ EE2 batch degradation tests, 500 mL aliquots of mixed liquor were collected at the end of the aerobic SBR period and transferred directly to 1-L flasks, immediately aerated with sparging stones and an aquarium pump, amended with 300 to 400 ng/L E1, E2, and EE2, and continuously mixed with magnetic stir bars. EE2 concentrations in the 500 mL aliquots were monitored for five hours throughout the Phase II batch tests, and for 12 hours throughout the Phase III batch tests. Example plots of EE2 concentrations obtained during batch degradation tests are given in Appendix A. The temperatures of the Phase I, Phase II, and Phase III EE2 batch degradation tests averaged 18.3°C, 24°C, and 20°C respectively.

Modeling EE2 Biodegradation and Solids Partitioning

EE2 biodegradation was assumed to obey a pseudo-first order kinetic model as a function of soluble estrogen and biomass VSS concentration.²⁵ Because the biomass concentration could not be determined directly, the MLVSS concentration was used as a surrogate measure for the amount of biomass.

$$\frac{dE_{tot}}{dt} = -k_b X_{VSS} E_{aq} \quad (1)$$

where: E_{tot} is the total EE2 concentration (ng/L), t is the time (days), k_b is the pseudo first-order EE2 biodegradation coefficient (L/g VSS/d), X_{VSS} is the MLVSS concentration (g VSS/L), and E_{aq} is the EE2 concentration in the bulk aqueous phase (ng/L). To determine values of k_b from EE2 batch degradation test data, the above equation was linearized as²⁵:

$$\ln\left(\frac{E_{tot}}{E_{tot,initial}}\right) = -k_b \left(\frac{X_{VSS}}{1 + K_p X_{VSS}}\right) \quad (2)$$

where: K_p is the EE2 solid-liquid partitioning coefficient (L/g VSS). Assumptions used in quantifying the first-order biodegradation coefficient (k_b) using the above model are described in detail by Gaulke et al.²⁵ Values of k_b were calculated by multiplying the slope of the linear trendline of $\ln(E_{tot}/E_{tot,initial})$ versus time by $(1+K_p X_{VSS})/X_{VSS}$.

The EE2 solid-liquid partitioning coefficient for AS biomass (K_p) was measured by simultaneously taking soluble and total EE2 measurements near the end of each batch degradation test. The specific sorbed concentration (E_{sorb} , ng/g) was determined by:

$$E_{sorb} = \frac{E_{tot} - E_{aq}}{X_{VSS}} \quad (3)$$

and the EE2 solid-liquid partitioning coefficient for AS biomass was then calculated as:

$$K_p = \frac{E_{sorb}(time = t)}{E_{aq}(time = t)} \quad (4)$$

One-way Analysis of Variance (ANOVA) and two-tailed Student's t-test were used to compare average EE2 k_b and K_p values between reactors.

Analytical Methods

Estrogen Stocks and Reagents: The estrogens, E1 ($\geq 99\%$), E2 ($\geq 98\%$), and EE2 ($\geq 98\%$), were purchased from Sigma Aldrich, and deuterated internal standards ($>98\%$), estrone-2,4,16,16-d₄ (d₄E1), 17 β -estradiol-2,4,16,16-d₄ (d₄E2) and 17 α -ethynylestradiol-2,4,16,16-d₄ (d₄EE2), were purchased from C/D/N Isotopes, Inc. Additional reagents used in the preparation and analysis of estrogens include: dansyl chloride ($\geq 99\%$) and formic acid purchased from Sigma Aldrich, HPLC grade acetonitrile (ACN) and methanol (MeOH) obtained from EMD Chemicals, and analytical grade sodium bicarbonate (NaHCO₃) purchased from EMD Chemicals. Calibration standards included 1, 5, 10, 25, 50, 100, 250, 500 and 1000 ng/L E1, E2, and EE2 solutions containing 100 ng/L of d₄E1, d₄E2, and d₄EE2 in a 1:1 MeOH:H₂O solution. A deuterated internal standard working solution of 1 μ g/L d₄E1, d₄E2, and d₄EE2 was prepared in a 1:1 MeOH:H₂O solution. Working solutions of 0.5 mg/L E1, E2, and EE2 were prepared in Milli-Q water. Calibration standards and working solutions were stored at 4°C. Estrogen and deuterated internal standard stock solutions were prepared in methanol and stored at -20°C. Prior to usage, all glassware was washed, soaked overnight in a sulfuric acid solution containing NOCHROMIX oxidizer, and rinsed three times with each Milli-Q water, acetone, and methylene chloride.

Estrogen Measurements: Estrogen measurements were prepared and conducted in accordance with the method described in detail by Gaulke et al.⁹ Samples for total estrogen measurements consisted of mixed liquor containing solid and liquid fractions. Mixed liquor samples for soluble estrogen measurements were centrifuged for 10 minutes at 3200 rpm and 4°C, after which the supernatant was decanted for analysis. Liquid-liquid extraction was performed on 0.5 mL sample aliquots by adding 3 mL of ethyl acetate and shaking vigorously for 10 minutes. The resulting organic fraction was thereafter transferred to a clean sample tube and evaporated to dryness at

40°C with nitrogen gas. Evaporated samples were then reconstituted with 100 µL of NaHCO₃ buffer (pH 10.5) and 100 µL of 1 mg/mL dansyl-chloride in ACN, and held for 30 minutes at 60°C prior to immediate analysis with LC-MS/MS.

Estrogen quantification was performed using a Shimadzu LC-20AD high performance liquid chromatograph (LC) (Kyoto, Japan) accompanied with Applied Biosystems 4000 Q-Trap tandem mass spectrometer (MS/MS) (Foster City, CA). Estrogen separation and elution with LC-MS/MS followed the scheme described by Gaulke et al.⁹ The limit of detection (LOD) and method limit of quantification (LOQ) for E1, E2, and EE2 was 5 ng/L. The natural estrogens, E1 and E2, were included in the feed to simulate a municipal wastewater estrogen matrix, yet were present below the LOD in all reactor effluents and were degraded too rapidly for the accurate determination of biodegradation kinetics during batch tests (data not shown).

Nutrient and Solids Measurements: Analysis of COD and NH₃-N was conducted using HACH kits and a HACH DR/4000 U Spectrophotometer (Loveland, CO) (Methods 8000 and 10023, respectively). Samples were filtered with 0.45 µm Supor membrane filter (Pall Sciences) prior to sCOD and NH₃-N analysis, and were filtered further with a 0.2 µm Supor membrane filter (Pall Sciences) prior to NO₃-N, NO₂-N, and PO₄-P analysis. A Dionex ICS-3000 Ion Chromatography System (Sunnyvale, California) was used to measure NO₃-N, NO₂-N, and PO₄-P. Analysis of TSS and VSS was conducted in accordance to Standard Methods²⁶, except that 1.2 µm glass-fiber filters (Whatman Grade GF/C) were used. An Oakton 500 pH probe and meter was used to measure pH.

Table 2. Summary of SBR Operational Parameters and Average Performance for the Three Experimental Phases. (Average values shown with standard deviation in parenthesis)

Parameter	Phase I SBRs			Phase II SBRs			Phase III SBRs			
SRT (d)	8.7			6.0			4.0			
Ambient Temp. (°C)	17.5 (1.7)			21.3 (1.7)			20.0			
Selector Process	High-F/M _f Aerobic	High-F/M _f Anoxic/Aerobic	High-F/M _f Anaer./Aerobic	High-F/M _f Aerobic	Low-F/M _f Aerobic	High-F/M _f Aerobic ^a	High-F/M _f Aerobic	Low-F/M _f Aerobic	Low-F/M _f Anoxic/Aerobic	
MLVSS (mg/L)	885 (87)	789 (65)	937 (97)	571 (55)	486 (62)	392 (46)	255 (37)	246 (24)	291(9)	
F/M _f ^b (g COD/g VSS/d)	10.2	11.4	9.6	15.3	0.7	15.0	84.5	1.5	1.2	
Reactor pH	7.2 (0.3)	7.5 (0.3)	7.3 (0.4)	7.4 (0.2)	7.3 (0.1)	7.3 (0.1)	7.8 (0.2)	7.6 (0.2)	7.8 (0.2)	
Influent (mg/L) (Includes synthetic feed addition)										
TCOD	375 (55) ^c			364 (30) ^c			244 (30)	265 (68)	291(103)	295 (106)
sCOD	230 (55)	234 (73)	250 (107)	236 (43)	226 (32)	119 (42)	164 (26)	163 (28)	161 (28)	
NH ₃ -N	27 (7.5)	26 (12)	27 (12)	25 (6.3)	29 (4.0)	24 (6.9)	23 (6.7)	24 (6.7)	24 (6.4)	
NO ₃ -N	0.1 (0.2)	9.8 (1.0)	0.1 (0.3)	2.2 (2.0)	0.3 (0.3)	1.3 (1.6)	0.3 (0.2)	0.7 (0.6)	25 (2.2)	
PO ₄ -P	6.5 (0.8)	6.4 (0.5)	6.5 (0.6)	7.7 (0.7)	7.4 (0.9)	7.3 (0.5)	1.6 (0.5)	1.7 (0.5)	1.7 (0.5)	
TSS	64 (12) ^c			58 (9.3) ^c			83 (22)	97 (17)	95 (17)	
VSS	57 (12) ^c			51 (7.4) ^c			70 (21)	83 (16)	81 (15)	
Effluent (mg/L)										
sCOD	21.8 (4.8)	22.0 (4.7)	21.0 (3.5)	22.2 (3.3)	22.0 (5.3)	21.5 (4.4)	30 (10)	34 (11)	32 (10)	
NH ₃ -N	0.05 (0.04)	0.05 (0.03)	0.05 (0.04)	0.04 (0.02)	0.09 (0.14)	0.03 (0.01)	0.04 (0.0)	0.08 (0.0)	0.05 (0.03)	
NO ₃ -N	14 (3.2)	12 (6.3)	5.4 (1.1)	24 (1.8)	20 (2.4)	29 (1.8)	15 (4.3)	16 (4.9)	28 (0.8)	
PO ₄ -P	5.2 (0.6)	5.0 (0.6)	2.0 (1.4)	6.9 (0.7)	7.2 (0.8)	7.1 (0.6)	1.5 (0.5)	1.6 (0.6)	1.4 (0.5)	
TSS	9.1 (13)	9.8 (6.0)	7.7 (5.7)	15 (7.4)	13 (6.8)	16 (8.7)	33 (9.8)	20 (8.0)	22 (6.7)	

Key: SRT=solids retention time; MLVSS=mixed liquor volatile suspended solids; F/M_f=initial feeding food-to-biomass ratio; TCOD=total chemical oxygen demand; sCOD=soluble chemical oxygen demand; NH₃-N=ammonia as nitrogen; NO₃-N=nitrate as nitrogen; PO₄-P=phosphate as phosphorus

^a Received a lower fraction of influent rbCOD

^b Initial feed condition F/M_f for SBR determined as²⁷: $F/M_f = (Q_{feed})(TCOD_{in})/(MLVSS \cdot V)$ where Q_{feed} is flow rate during feeding (L/d), $TCOD_{in}$ is average influent TCOD (mg COD/L), and $MLVSS \cdot V$ is the average reactor mixed liquor volatile suspended solids mass (mg VSS)

^c Influent feed parameters measured as composite averages for multiple reactors

RESULTS AND DISCUSSION

Metabolic selectors were operated during Phase I at high initial substrate loading to compare the EE2 biodegradation abilities of distinct AS microbial communities that express rapid uptake of rbCOD under aerobic, anoxic or anaerobic redox conditions. The aim of the Phase II reactors was to compare the EE2 biodegradation activities of AS biomass that was subject to kinetic population selection at high- F/M_f loading and AS biomass that was adapted to growth at low substrate concentrations with low- F/M_f feeding. The Phase III reactors were operated to examine the relative effects of kinetic and metabolic selection by comparing the EE2 biodegradation activities of AS biomass populations grown at low substrate concentration under aerobic and anoxic/aerobic conditions, as well as AS biomass grown aerobically at high initial substrate loading. The impacts of metabolic and kinetic population selection conditions on EE2 biodegradation activity were determined by comparing pseudo first-order biodegradation rate coefficients (k_b) among the different AS systems.

EE2 Biodegradation Kinetics in High- F/M_f Metabolic Selectors

The metabolic selectors operated in Phase I achieved their expected EBPR and nitrogen removal performances (Table 2) and removed a substantial portion of soluble COD (sCOD) during the metabolic selector fill/react time. The average removal of influent sCOD was 93% and 95% ($n=17$) in the anoxic/aerobic and anaerobic/aerobic reactors, respectively, during their 1.5-hour metabolic selection periods. The anoxic/aerobic selector removed an average of 14.7 mg $\text{NO}_3\text{-N/L}$ relative to the aerobic selector ($n=17$) (Table 2), and had an average COD/ $\text{NO}_3\text{-N}$ consumption ratio of 7.4 during the anoxic period ($n=17$). The anaerobic/aerobic selector removed an average of 260% more influent phosphorous (3.2 mg P/L) than the aerobic selector

($n=23$) (Table 2). Therefore, the different redox growth conditions of the Phase I AS metabolic selectors likely resulted in distinct heterotrophic communities, as indicated by their respective nutrient removal performances.

Despite the different biological nutrient removal processes observed in the Phase I metabolic selectors, no statistically significant difference was detected in their EE2 biodegradation kinetics (Figure 1). One-way ANOVA on the average pseudo first-order EE2 biodegradation rate coefficients (k_b) of the Phase I SBRs revealed a p level of 0.095. Though the aerobic, anoxic/aerobic, and anaerobic/aerobic SBRs in Phase I had different metabolic selection processes, the reactors were subject to similar kinetic selection processes due to their relatively high F/M_f feeding conditions (Table 2). The similar EE2 biodegradation kinetics observed in the Phase I selectors thus could have been caused by their high- F/M_f kinetic selection processes or by their different redox conditions under which rbCOD was consumed, or by both of these population selection mechanisms collectively. Therefore, the type of metabolic selection process did not appear to significantly impact EE2 biodegradation kinetics in AS systems that employed high substrate concentrations in the initial contact zone.

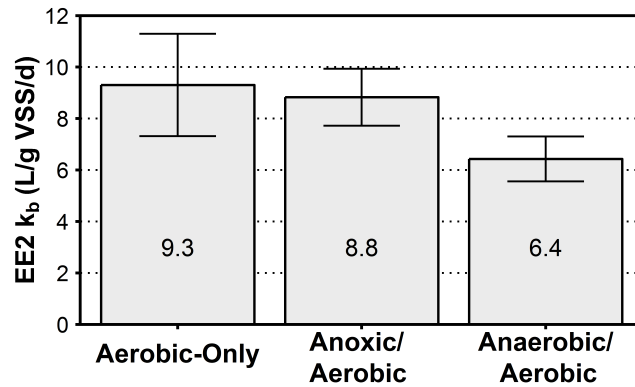


Figure 1. Average pseudo first-order EE2 biodegradation rate coefficients (k_b) for batch degradation tests (average temperature of 18.3°C) with activated sludge from the Phase I metabolic selector SBRs that were operated at high initial food-to-biomass-ratio (F/M_f) feeding conditions and an 8.7-day SRT. Average EE2 k_b of the Phase I SBRs were not statistically different at the $p=0.05$ level. ($n=3$ for each SBR. Average EE2 k_b values shown in center of bars. Error bars indicate one standard deviation.)

Impact of Kinetic Selection on EE2 Biodegradation Rate Coefficients

In order to test the effects of kinetic population selection on EE2 biodegradation activity in aerobic AS systems, the duration of feeding was adjusted in the Phase II reactors to alter their initial feeding F/M_f conditions (Table 2) and promote substrate uptake under concentrations that favored K-strategists in one SBR and r-strategists in two other SBRs. In this manner, an aerobic low- F/M_f reactor and two high- F/M_f aerobic selectors were operated in parallel. One of the high- F/M_f aerobic SBRs was operated with a lower portion of rbCOD in its influent feed to determine if high- F/M_f kinetic selectors would achieve similar EE2 k_b values with different wastewater compositions. The different initial contact F/M_f conditions employed in the reactors altered the substrate concentration available during microbial growth, which resulted in differing sludge morphologies in the high- F/M_f selectors and low- F/M_f reactor (Figure 2). The growth of K-strategist heterotrophs in the low- F/M_f reactor was apparent with the dominant presence of filamentous microorganisms (Figure 2b), while the two high- F/M_f selectors achieved non-

filamentous sludge flocs typical of r-strategist microorganisms²³ (Figures 2a and 2c). This observation was in agreement with the kinetic selection theory proposed by Chudoba et al.²⁴, in which filamentous microorganisms with lower half-saturation coefficients (K-strategists) are enriched in CMAS reactor configurations, while non-filamentous r-strategists are dominant in batch fed kinetic selector SBRs²⁸⁻³⁰. Moreover, F/M ratios of 12 to 14 g COD/g MLVSS/d are suggested for initial contact zones of aerobic selectors²³, which are similar to the F/M_f values applied in the Phase II high- F/M_f selectors (Table 2). Thus, the different sludge morphologies observed in the Phase II aerobic low- F/M_f reactor and high- F/M_f aerobic selectors indicate that their heterotrophic microbial populations likely diverged due to the different kinetic growth selection processes applied.

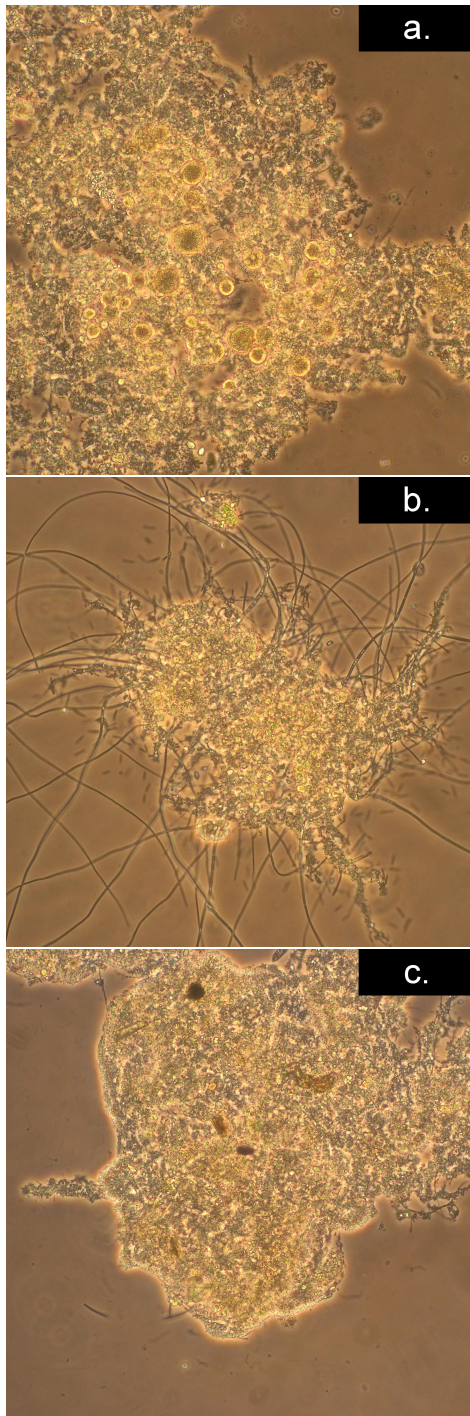


Figure 2. Photomicrographs of Phase II SBR activated sludge flocs with phase contrast and 200X magnification: (a) high- F/M_f aerobic selector, (b) aerobic low- F/M_f reactor, (c) high- F/M_f aerobic selector with low influent rbCOD.

A statistically significant increase in the first-order EE2 biodegradation rate coefficient (k_b) was associated with the low-F/M_f reactor relative to the two high-F/M_f selectors (Figure 3) ($p=0.002$ with one-way ANOVA). Comparisons using a two-tailed Student's t-test revealed that the average EE2 k_b of the low-F/M_f reactor was significantly greater than the high-F/M_f selector operated with the same influent wastewater ($p=0.01$) as well as the high-F/M_f selector operated with less rbCOD in the feed ($p=0.02$). However, no statistically significant difference was detected between the EE2 k_b values of the two high-F/M_f aerobic selectors ($p=0.15$), despite their different fractions of influent rbCOD (Table 2). These results suggest that EE2-degrading populations can grow on rbCOD at the low concentrations promoted in low-F/M_f feeding conditions, but they may be outcompeted for rbCOD at the high concentrations applied in high-F/M_f operating conditions. The similar EE2 k_b values observed in the aforementioned Phase I high-F/M_f metabolic selectors (Figure 1) may thus be a result of similar EE2 biodegradation kinetics in r-strategist populations of aerobic bacteria, denitrifying bacteria, and PAOs, which consumed a majority of the influent soluble growth substrate at a high concentration. An additional possibility is that EE2-degrading populations grow on cellular endogenous decay products in high-F/M_f systems due to the rapid rbCOD substrate uptake by non-EE2-degrading r-strategists during the fill/react period. Yet, the increased EE2 k_b in the Phase II low-F/M_f reactor (Figure 3) was not likely attributed to enhanced growth on endogenous decay products, as the low-F/M_f reactor and one high-F/M_f reactor treated the same fraction of influent rbCOD and were operated with identical SRTs, and thus had similar fractions of endogenous decay products.

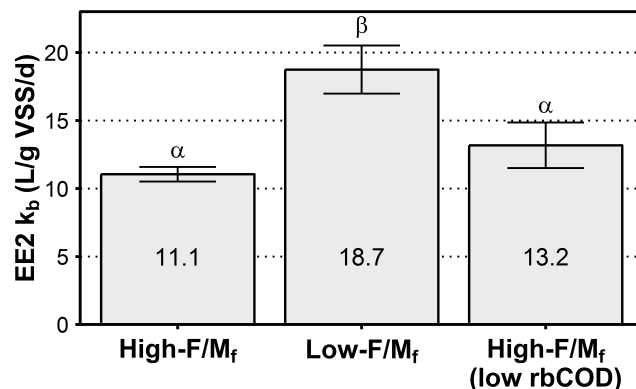


Figure 3. Average pseudo first-order EE2 biodegradation rate coefficients (k_b) for batch degradation tests (average temperature of 24.0°C) with activated sludge from the Phase II kinetic selector SBRs, operated at a 6-day SRT and either high or low initial food-to-biomass-ratio (F/M_f) feeding conditions. The α and β symbols indicate statistically significant different EE2 k_b values ($p < 0.05$). ($n=3$ for each SBR. Average EE2 k_b values shown in center of bars. Error bars indicate one standard deviation.)

The highest EE2 first-order biodegradation rate coefficient of the Phase II SBRs was associated with the lowest initial growth substrate concentration in the low-F/M_f reactor (Figure 3, Table 2). These results suggest that kinetic population selection is an important operational factor governing the growth and activity of EE2-degrading microorganisms in AS. Similarly, Koh et al.¹⁸ hypothesized that observed differences in EE2 biomass biodegradation activities between a nitrification/denitrification WWTP and a nitrification/denitrification/P-removal WWTP were attributed to the growth of K-strategist heterotrophic populations that scavenge low concentrations of substrate. However, the F/M ratios employed in the Koh et al.¹⁸ study only varied by 0.05 g BOD/g MLVSS/d, which limited the scope of assessing kinetic selection effects on EE2 biodegradation activity. In this study, the initial feed F/M_f ratio in the Phase II low-F/M_f reactor was reduced by approximately 21-fold (14.3 g COD/g MLVSS/d) compared to the values for the high-F/M_f selectors operated in parallel (Table 2), which resulted in an increase in the EE2 k_b of the low-F/M_f reactor by a factor of 1.4 to 1.7 (Figure 3). This finding supports the

postulation of greater estrogen removal activities for AS mixed liquor grown in longer SRT and lower F/M systems^{16,22} by identifying kinetic population selection at low growth substrate concentration as an important mechanism enhancing the EE2 biodegradation activity of AS populations.

Increased estrogen biodegradation observed in AS systems operated at longer SRTs and lower F/M ratios^{16,22} has also been thought to be attributed to the growth of nitrifying organisms³¹⁻³⁶. However, the growth conditions at longer SRTs and lower F/M that may support nitrifying organisms in AS similarly promote the growth of specialist heterotrophs capable of scavenging a wide range of organic compounds.³⁷ In the Phase II reactors, the highest fraction of active nitrifying biomass was 3.9%, which occurred in the high-F/M_f selector with lower influent rbCOD, while the low-F/M_f reactor and the high-FM_f selector that were operated with the same influent wastewater had active nitrifying biomass fractions of 1.8% and 2.2%, respectively (Appendix A). The highest EE2 k_b occurred in the AS configuration with the lowest active nitrifying biomass fraction, and thus the increased EE2 k_b in the low-F/M_f reactor was not correlated with nitrifying activity and was therefore more likely attributed to heterotrophic bacterial populations. The growth of filamentous K-strategist heterotrophs is supported predominantly in AS systems with F/M ratios below 1.5 g COD/g MLVSS/d²³, which applies to the Phase II low-F/M_f reactor (Table 2) in which filamentous growth was observed (Figure 2b). While the number of cultured EE2-degrading microorganisms is currently few, various EE2-degrading K-strategist heterotrophs have been isolated from AS systems. For instance, EE2-degrading strains of *Rhodococcus equi* and *R. erythropolis* were isolated from AS systems in Japanese WWTPs.¹¹ *Rhodococcus* species are members of the mycolata taxon within the class of *Actinobacteria*, and have been identified as filamentous³⁷ and foam-causing microorganisms³⁸⁻⁴¹

in AS systems, thus suggesting their K-strategist growth regime. In addition, *Rhodococcus* have been shown to degrade EE2 more rapidly than other heterotrophs from the genera *Bacillus* and *Pseudomonas*.⁴² While it remains unclear if all K-strategist heterotrophs generally have enhanced EE2 degradation activity relative to other heterotrophs, this research suggests that improved EE2 biodegradation kinetics are observed in AS systems applying kinetic population selection toward aerobic K-strategist heterotrophic populations using low substrate growth conditions.

Combined Effects of Kinetic and Metabolic Selection Processes on EE2 Biodegradation

The purpose of the Phase III SBR operation was to compare the relative impacts of kinetic and metabolic selection processes on EE2 biodegradation kinetics in AS populations. An aerobic low-F/M_f reactor was operated in parallel with an anoxic/aerobic low-F/M_f reactor to examine the effects of metabolic selection in environments adapted for K-strategists. A high-F/M_f aerobic selector was also operated to validate kinetic population selection effects with different AS inoculum and primary effluent sources than the Phase II reactors. The anoxic/aerobic low-F/M_f reactor achieved its target BNR performance by averaging 95% sCOD removal during its anoxic fill/react period ($n=6$), and operated with an average sCOD/NO₃-N removal ratio of 5.5 during the anoxic fill/react period ($n=7$). As F/M_f ratios above 1.8 g COD/g MLVSS/d are used to prevent filamentous growth in anoxic selectors²³, the Phase III anoxic/aerobic low-F/M_f reactor likely promoted the growth of facultative K-strategist heterotrophs capable of denitrification by employing a F/M_f ratio of 1.2 g COD/g MLVSS/d (Table 2). The aerobic low-F/M_f reactor and high-F/M_f aerobic selector likely supported aerobic K-strategist and r-strategist heterotrophs, respectively.

A statistically significant difference was observed in the first-order EE2 biodegradation rate coefficients (k_b) of the Phase III SBRs ($p=0.00009$ with one-way ANOVA). The EE2 k_b of the aerobic low-F/ M_f reactor was statistically greater than the high-F/ M_f aerobic selector operated in parallel ($p=0.0002$ with Student's t-test) (Figure 4), with a corresponding 2.2-fold increase in EE2 k_b . This result validates the trend of higher EE2 biodegradation rate coefficients for aerobic low-F/ M_f operating conditions observed in the Phase II experiment (Figure 3). However, the EE2 k_b of the Phase III anoxic/aerobic low-F/ M_f reactor was not significantly different than the high-F/ M_f aerobic selector ($p=0.06$ with Student's t-test). This finding suggests that metabolic selection may have countered the effects of low substrate kinetic selection in the anoxic/aerobic low-F/ M_f reactor, thus reducing its EE2 biodegradation activity to the level of the high-F/ M_f aerobic selector. Additionally, the statistically significant increase in the EE2 k_b of the aerobic low-F/ M_f reactor relative to the anoxic/aerobic low-F/ M_f reactor ($p=0.003$ with Student's t-test) of 1.7-times (Figure 4) further suggests that metabolic selection may impact the activity of EE2-degrading microorganisms in AS. Prior investigations have indicated that EE2 biodegradation is severely hindered under anoxic conditions relative to aerobic conditions^{17,43-46}, yet differences in the aerobic EE2 biodegradation kinetics of biomasses adapted to anoxic or aerobic conditions have remained unclear. To the best of our knowledge, this is the first reported difference in aerobic EE2 biodegradation rate coefficients between anoxic/aerobic and aerobic AS systems operated with identical SRT, HRT, influent wastewater, and feeding regime. This trend was observed only in the low substrate growth condition reactors in Phase III, however, and not in the aforementioned Phase I high-F/ M_f metabolic selectors. Despite the different feeding strategies applied to the anoxic/aerobic selectors in Phases I and III, their EE2 k_b values were both similar to the high-F/ M_f aerobic selectors operated in parallel, while the only observed significant

increases in EE2 k_b occurred in aerobic low-F/M_f systems. Therefore, it appears that both high substrate and anoxic growth conditions hinder EE2 biodegradation kinetics of AS populations relative to aerobic growth conditions at low substrate concentration.

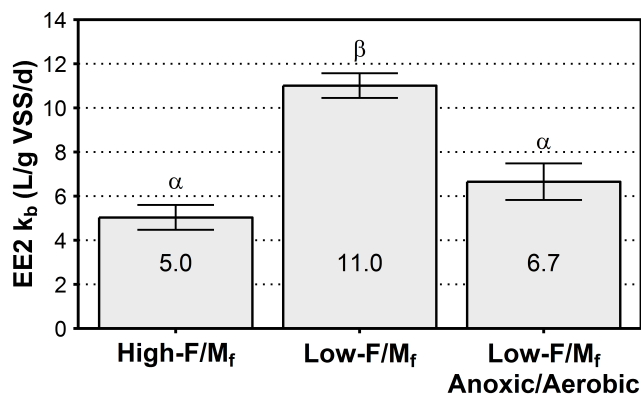


Figure 4. Average pseudo first-order EE2 biodegradation rate coefficients (k_b) for batch degradation tests (average temperature of 20.0°C) with activated sludge from the Phase III kinetic and metabolic selector SBRs operated at a 4-day SRT and either high or low initial food-to-biomass-ratio (F/M_f) feeding conditions. The α and β symbols indicate statistically significant different EE2 k_b values ($p < 0.05$). ($n = 3$ for each SBR. Average EE2 k_b values shown in center of bars. Error bars indicate one standard deviation.)

Effect of AS Reactor Configuration on EE2 Partitioning to Biosolids

It is hypothesized that the kinetic and metabolic selective pressures applied in the AS reactor configurations in this study resulted in divergent microbial communities with distinct physiological properties. EE2 solid-liquid partitioning coefficient (K_p) values of the low-F/M_f reactor biomasses in Phases II and III were greater than for the biomasses of the parallel high-F/M_f selectors (Table 3), suggesting divergence in their microbial community physiologies. This effect was highly significant in Phase III ($p = 8.0E-7$ with one-way ANOVA), as the average EE2 K_p values of the aerobic and anoxic/aerobic low-F/M_f reactor biomasses were significantly greater than the high-F/M_f aerobic selector biomass ($p = 5.0E-6$, $6.0E-5$, respectively, with Student's t-test). The EE2 K_p values of the two low-F/M_f reactor biomasses in Phase III were not,

however, significantly different ($p=0.88$ with Student's t-test), suggesting similar biomass characteristics in the two systems dominated by K-strategist heterotrophs. Filamentous heterotrophic bacteria, including identified EE2-degrading *Rhodococcus* species^{11,12,42} belonging to the mycolic-acid containing *Actinobacteria*, can have strongly hydrophobic cell-wall properties^{37,39,41}, which may be a competitive strategy for growth on hydrophobic substrates in activated sludge⁴⁷. A greater EE2 distribution coefficient (K_d) has been reported for a membrane bioreactor (MBR) biomass relative to SBR biomasses, and was attributed to greater floc surface area and cell hydrophobicity in the MBR sludge.⁴⁸ Mean floc particle size and specific surface area were also shown to have a non-linear effect on the EE2 K_d of AS biomass; with larger K_d values associated with smaller mean floc particle sizes and correspondingly higher specific surface areas.⁴⁹ Thus, higher EE2 partitioning to biomass may occur in AS systems operating with lower organic substrate concentrations possibly due to the growth of filamentous K-strategist heterotrophs with hydrophobic cell characteristics and greater specific floc surface areas.

The calculation of pseudo first-order EE2 biodegradation coefficients (k_b) using Equation 2 relies on the EE2 solid-liquid partitioning coefficient (K_p). A sensitivity analysis of the pseudo first-order EE2 biodegradation model revealed that a 25% increase in the EE2 K_p value of the Phase III aerobic low-F/ M_f reactor resulted in a 3% increase in its average EE2 k_b . Therefore, the higher EE2 biodegradation rate coefficients observed in the aerobic low-F/ M_f AS systems in this study were not explained by elevated EE2 K_p values.

Table 3. Average Solid-Liquid EE2 Partitioning Coefficients (K_p) for the SBR Activated Sludge from Each Experimental Operating Phase. (Average values shown with standard deviation in parenthesis)

Selector Process	Phase I SBRs ($n=4$)			Phase II SBRs ($n=5$)			Phase III SBRs ($n=6$)		
	High-F/ M_f Aerobic	High-F/ M_f Anoxic/ Aerobic	High-F/ M_f Anaer./ Aerobic	High-F/ M_f Aerobic	Low-F/ M_f Aerobic	High-F/ M_f Aerobic ^a	High-F/ M_f Aerobic	Low-F/ M_f Aerobic	Low-F/ M_f Anoxic/ Aerobic
EE2 Solids-Liquid Partitioning (K_p) (L/kg-VSS)	554 (112)	684 (62)	408 (57)	405 (163)	682 (203)	440 (154)	303 (138)	867 (76)	856 (142)

Key: SBR=sequencing batch reactor; F/ M_f =initial feeding food-to-biomass ratio
^a Received lower fraction of influent rbCOD

EE2 Removal Efficiency and Biodegradation Kinetics in Relation to AS Reactor Configuration

The average EE2 removal efficiencies for the three experimental Phases ranged from 41% to 87% (Table 4), which are within the range of reported EE2 removals in full-scale AS WWTPs^{14,16-18}. While operation under aerobic low substrate growth conditions contributed to an increase in EE2 biodegradation kinetics in the Phase II and III reactors (Figures 3 and 4), the same trend was not observed in their EE2 removal efficiencies (Table 4). The lack of correlation between EE2 biodegradation kinetics and removal efficiency can be explained by the different hydraulic characteristics of the low-F/ M_f and high-F/ M_f reactor configurations. The hydraulic regimes of the high-F/ M_f AS systems were similar to plug flow reactor (PFR) conditions, in which the rapid fill-time (Table 1) promoted batch reactor kinetics with a high initial EE2 concentration that decreased during the remaining aeration period. Higher EE2 removal rates occur at higher EE2 concentrations due to the first-order mode of its biodegradation (Equation 1). On the other hand, the low-F/ M_f systems had a relatively constant low EE2 concentration due

to the semi-continuous feeding throughout the react period. Likewise, longer reaction times are needed in a continuously stirred tank reactor (CSTR) in order to achieve a similar effluent concentration as in a PFR operated with the same first-order reaction rate coefficient.⁵⁰ Despite the 1.7-fold increase in the first-order EE2 k_b of the Phase II low-F/ M_f reactor (Figure 3), its semi-continuous feeding and consequent removal at a lower concentration resulted in an overall EE2 removal efficiency that was similar to the batch fed high-F/ M_f aerobic selectors operated in parallel (Table 4). Similarly, the 2.2-fold increase in EE2 k_b in the Phase III aerobic low-F/ M_f reactor relative to the high-F/ M_f selector (Figure 4) did not result in a significantly greater EE2 removal efficiency (Table 4). A tradeoff therefore exists between improved EE2 biodegradation kinetics and less favorable reactor hydraulic characteristics in continuously fed AS systems that operate with a constantly low substrate concentration.

Table 4. Average Total EE2 Concentrations and EE2 Removal Efficiencies Based on Samples at the End of the SBR Cycle for Experimental Phases I, II, and III. (Samples taken during the SBR aeration period within five minutes before settling. Average values shown with standard deviation in parenthesis)

	Selector Process	Average End-of-Cycle Total EE2 Concentration (ng/L) ^a	Average EE2 Removal Efficiency (%) ^{a,b}
Phase I SBRs (n=5)	High F/M _f Aerobic	29 (9.4)	81 (6.5)
	High F/M _f Anoxic/ Aerobic	24 (4.8)	83 (3.2)
	High F/M _f Anaerobic/ Aerobic	30 (5.2)	80 (3.3)
Phase II SBRs (n=7)	High-F/M _f Aerobic Selector	16 (2.3)	88 (1.4)
	Low-F/M _f Aerobic	20 (1.1)	85 (1.0)
	High-F/M _f Aerobic Selector ^c	18 (2.1)	80 (3.0)
Phase III SBRs (n=9)	High-F/M _f Aerobic Selector	153 (34)	52 (4.2)
	Low-F/M _f Aerobic	139 (20)	57 (3.8)
	Low-F/M _f Anoxic/ Aerobic	189 (28)	42 (6.8)

Key: SBR=sequencing batch reactor; F/M_f=initial feeding food-to-biomass ratio

^a Samples averaged over last 1.5 SRTs of operation

^b Removal efficiency calculated as: $(1 - EE2_{\text{end-of-cycle}} / EE2_{\text{influent}})$

^c Received lower fraction of influent rbCOD

This study shows that AS systems operating aerobically with low organic substrate concentrations can achieve microbial populations with greater specific EE2 biodegradation kinetics than AS selectors operating with high initial substrate concentrations. However, the EE2 removal efficiency of low- F/M_f systems is hindered by the lack of hydraulic staging relative to the PFR designs used to implement high- F/M_f kinetic population selection. Therefore, optimizing AS selector configurations to create favorable microbial biodegradation kinetics and efficient reactor hydraulic characteristics may result in improved EE2 removal in WWTPs. This could be accomplished by using larger initial contact AS reactors than are commonly implemented in order to promote microbial growth at a relatively low substrate concentration while also achieving a favorable reactor configuration with hydraulic staging.

CONCLUSIONS

A series of experiments were conducted with bench-scale AS SBRs that were fed domestic wastewater supplemented with acetate and estrogen compounds and operated with different metabolic and kinetic selector operating conditions in order to observe the impact of AS microbial population selection on EE2 biodegradation kinetics. The major findings from the experiments are summarized as follows:

- EE2 biodegradation activities in AS systems were impacted by metabolic and kinetic population selection mechanisms, suggesting that not all microbial populations degrade EE2 at the same specific rate, and thus microbial community composition is an important factor governing EE2 removal in AS WWTPs.
- Aerobic growth conditions under low organic substrate concentration selected for AS populations with the highest EE2 k_b values, indicating that growth conditions more favorable to aerobic K-strategists microbial populations result in higher EE2 biodegradation kinetics.
- Metabolic population selection can impact EE2 biodegradation, as an anoxic/aerobic selector that was operated at low-F/ M_f feeding conditions had an EE2 k_b that was 40% lower than a parallel low-F/ M_f aerobic reactor. However, the type of metabolic selection did not affect EE2 biodegradation kinetics in AS systems operated at high-F/ M_f feeding conditions.
- Greater EE2-biomass partitioning coefficients (K_p) were observed in AS reactors that were operated at low-F/ M_f feeding conditions.
- Although EE2 biodegradation kinetics were improved in aerobic reactors operating with low-substrate and complete-mix hydraulic regimes, their overall EE2 removal

efficiencies were similar to that of aerobic plug flow reactors that had lower EE2 biodegradation kinetics. Therefore, a tradeoff was observed between improved EE2 biodegradation kinetics and less favorable reactor hydraulic characteristics in the low- F/M_f AS systems that operated with a constantly low substrate concentration.

- An optimal AS reactor configuration to provide the highest EE2 removal efficiency is a series of staged aerobic reactors with a large enough first stage (low enough F/M_f) to promote increased EE2 biodegradation kinetics along with more favorable reactor hydraulic characteristics.

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APPENDIX A

The Appendix A section includes two equations and one graph.

A-1. Calculation of Active Nitrifying Biomass

The fraction of nitrifying biomass to the total active biomass was determined by calculating active heterotrophic and nitrifying biomass concentrations with the following two equations:

$$X_H = \frac{Y_H \cdot COD_{in} \cdot SRT}{HRT \cdot (1 + k_d \cdot SRT)} \quad \text{Equation A1}$$

where: X_H is the active heterotrophic biomass (mg/L), COD_{in} is the influent total COD (mg/L), Y_H is the heterotrophic biomass yield (0.4 mg VSS/mg COD), k_d is the heterotrophic decay coefficient (0.088 d⁻¹)

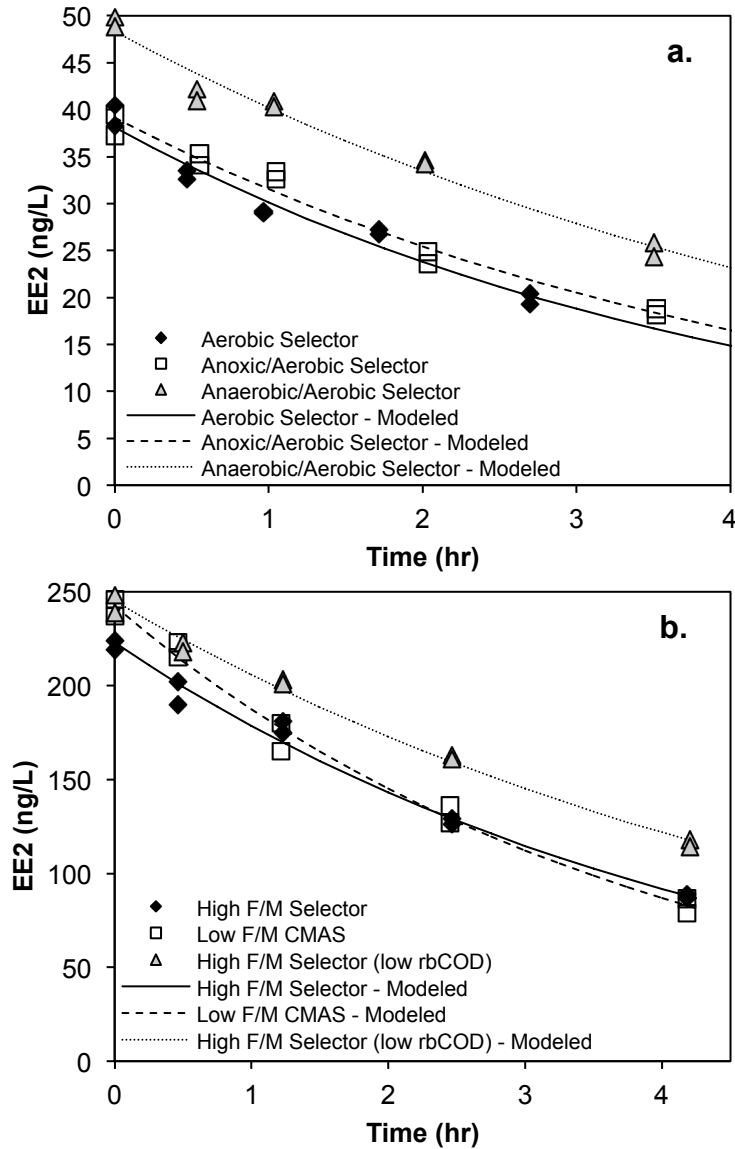
$$X_N = \frac{Y_N \cdot NO_{3out} \cdot SRT}{HRT \cdot (1 + k_{d_n} \cdot SRT)} \quad \text{Equation A2}$$

where: X_N is the active nitrifying biomass, NO_{3out} is the effluent nitrate concentration (mg-N/L) and represents oxidized ammonia, Y_N is the nitrifying biomass yield (0.12 mg VSS/mg COD), k_{d_n} is the nitrifying decay coefficient (0.06 d⁻¹).

The values of COD_{in} , NO_{3out} , SRT , and HRT for the Phase II reactors are given in Table 2. The fraction of active nitrifying biomass was calculated as $X_N/(X_N+X_H)$.

A-2. EE2 Batch Degradation Test Plots

The following figure shows total EE2 concentration profiles during individual batch tests in the three SBR operational Phases. Total EE2 concentrations modeled with the calculated pseudo first-order biodegradation rate coefficients from each batch test are also shown for model verification, with initial concentrations calculated from the intercept of $\ln(E_0/E)$ versus time.



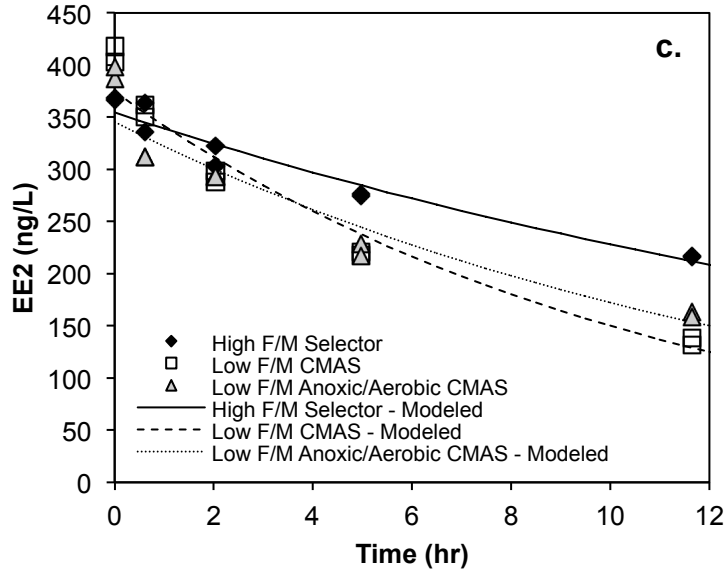


Figure A 1. Total EE2 concentrations throughout batch degradation tests for (a) Phase I metabolic selector SBRs, (b) Phase II kinetic selector SBRs, (c) Phase III kinetic and metabolic selector SBRs. (Observed data is shown with duplicate EE2 measurements at each time point.)