Testing the Gamma hypothesis

for two different hurdles, pH and undissociated acid concentration

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6th ICPMF, Washington

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Outline

- Introduction
  - Hurdle technology
  - Gamma hypothesis
- Material and Methods
- Results
  - Experiments
  - Modelling
- Conclusions

Hurdle technology

- Combinations of hurdles, used to achieve an overall level of protection in food (Leistner & Gorris, 1995)
  - Temperature
  - pH
  - A_w
- Advantages hurdle technology:
  - microbial stability
  - improved organoleptic properties/ increased consumer acceptability

Hurdle technology

Two views on hurdle technology

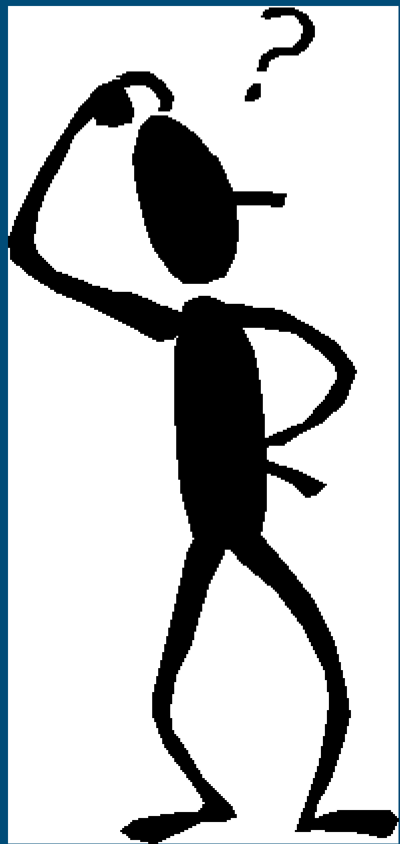
- Synergy: interactive effects, giving a greater protection than expected
- No synergy: environmental factors combine in a multiplicative manner to produce the observed overall microbial inhibition
- Gamma hypothesis

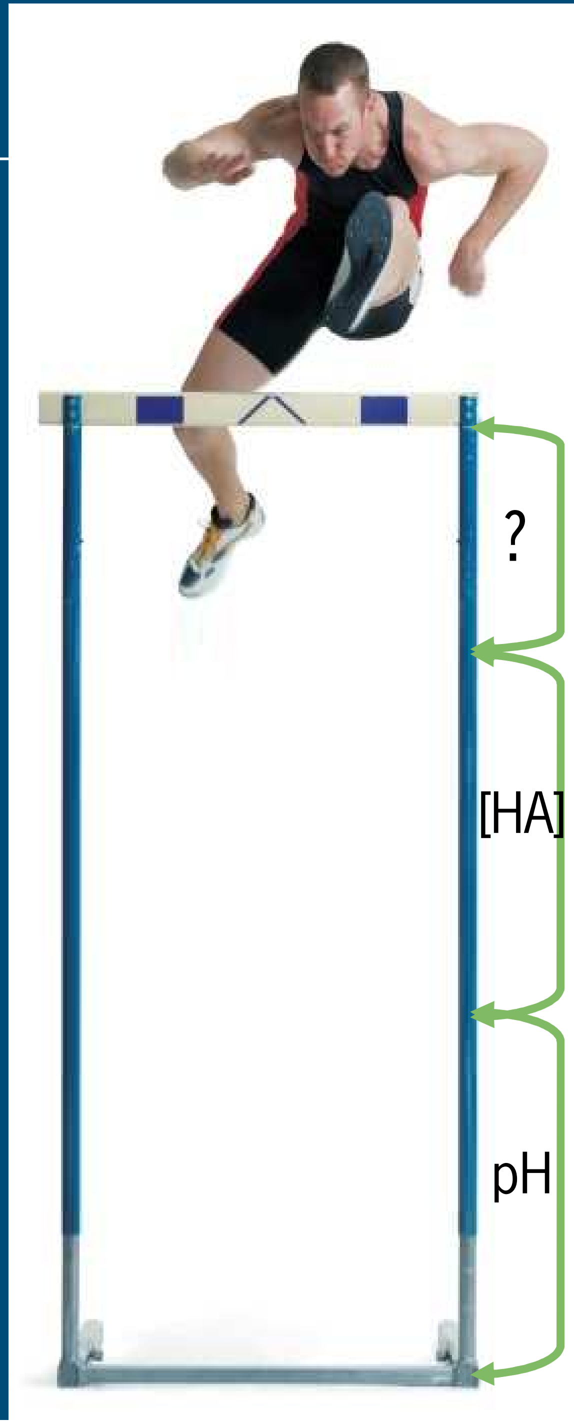
(Zwietering, Wijtzes, De Wit, Van 't Riet, 1992)

Gamma hypothesis

- For every hurdle a Gamma (γ) factor is calculated:
  - γ (T) $= \mu_{max} / \mu_{opt}$
  - γ (pH) $= \mu_{max} / \mu_{opt}$
  - γ (A_w) $= \mu_{max} / \mu_{opt}$
- Gamma hypothesis:
  - $\mu_{max}(T, pH, A_w) = \mu_{opt} * \gamma(T) * \gamma(pH) * \gamma(a_w)$

Synergy?

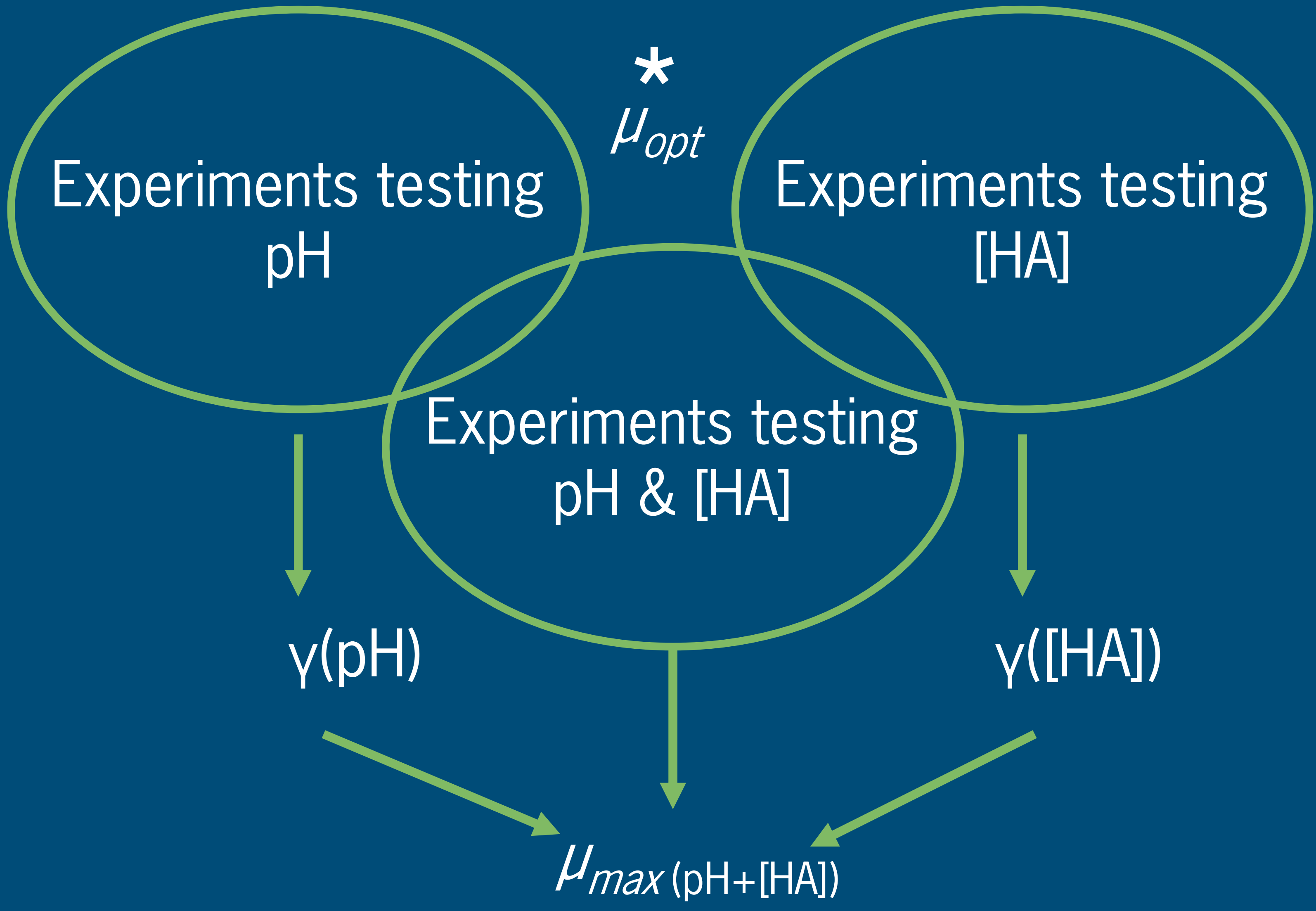




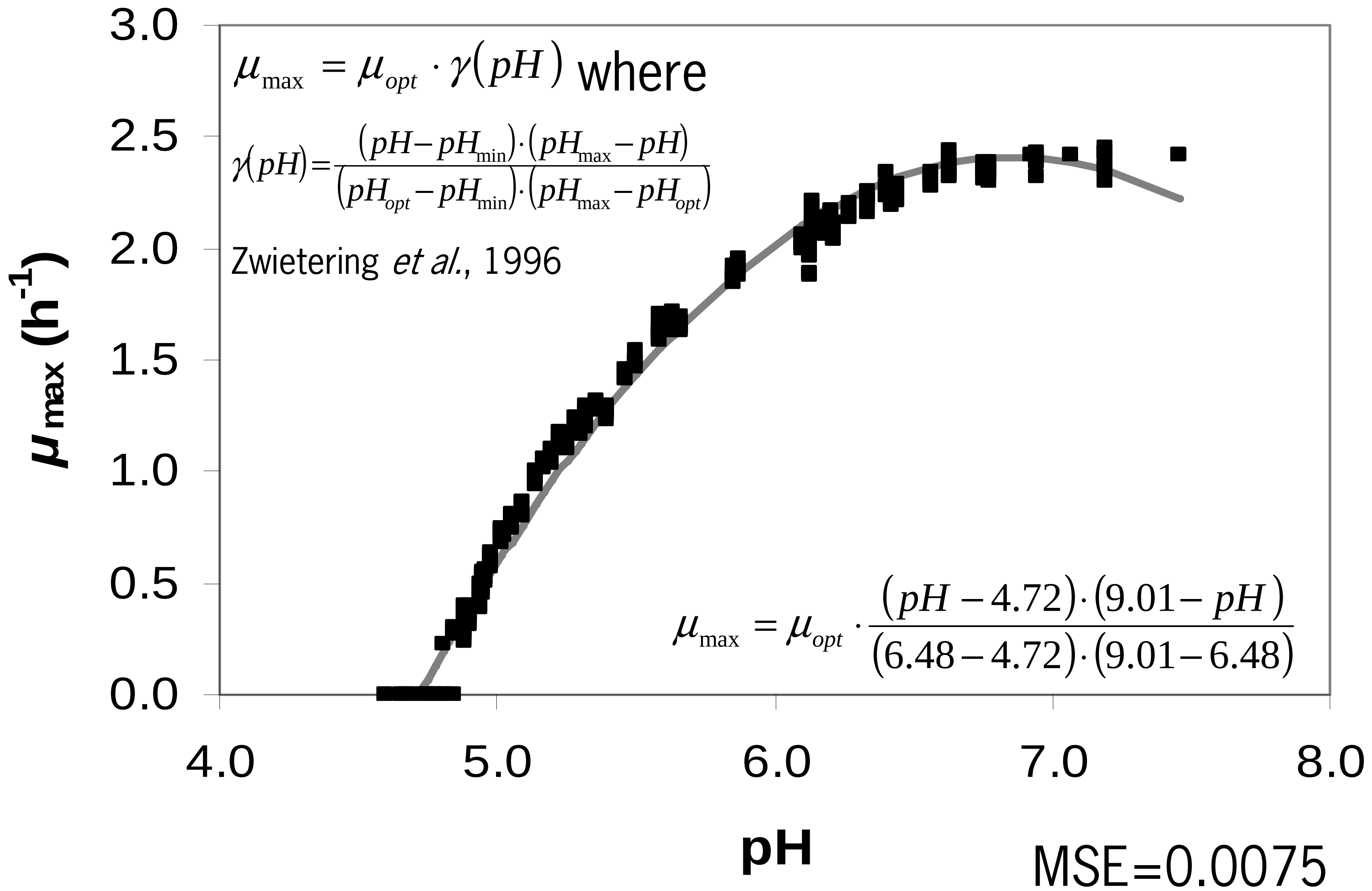
Experimental conditions

- *Bacillus cereus* F4810/72
- μ_{max} obtained using the OD measurements and *RRD* method
- (combined) effect of:
  - pH (sulfuric acid, H_2SO_4)
  - Undissociated acid concentration (formic acid, HFo)

Experimental setup

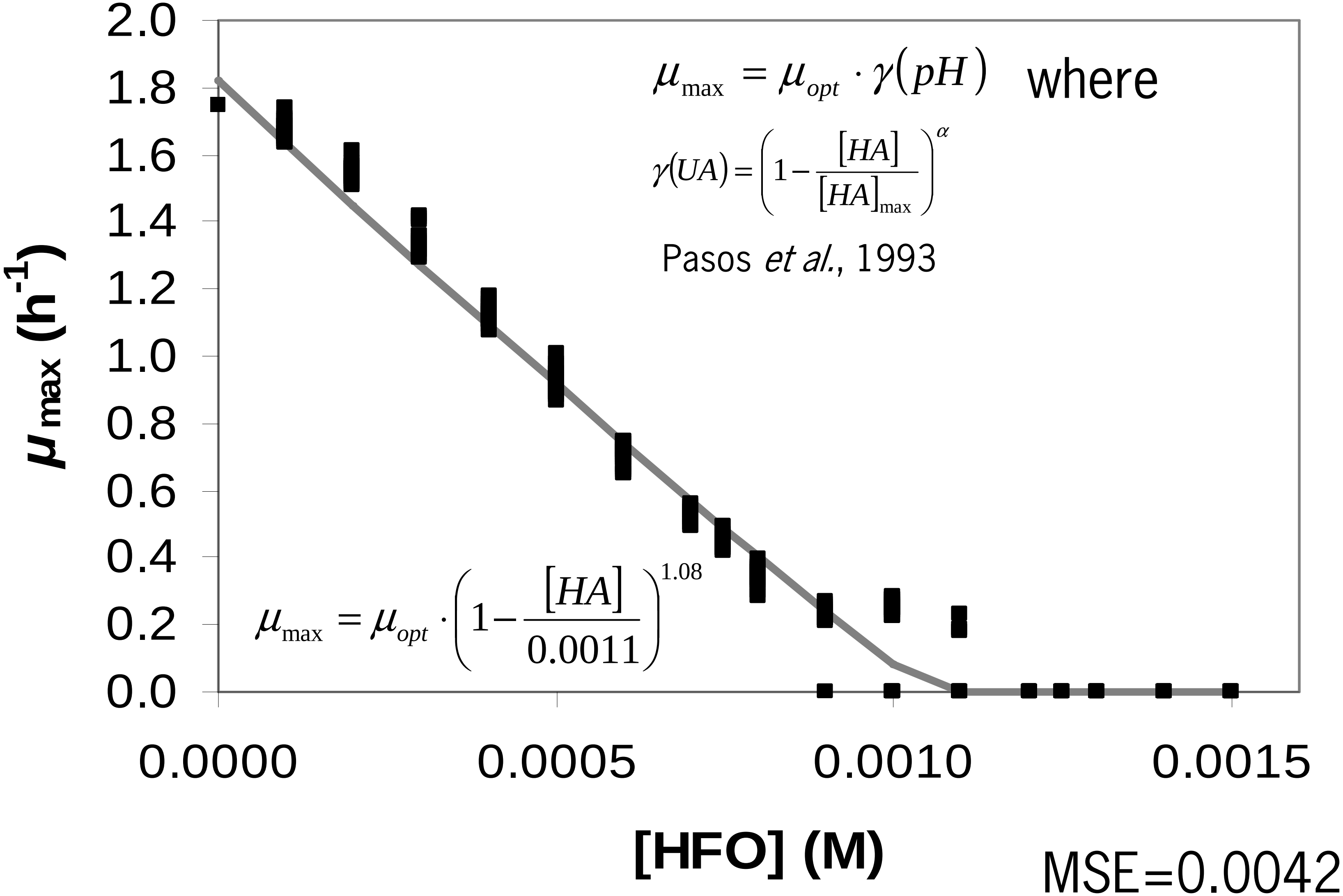


γ (pH)



$\mu_{max} = \mu_{opt} \cdot \gamma(pH)$ where
$\gamma(pH) = \frac{(pH - pH_{min}) \cdot (pH_{max} - pH)}{(pH_{opt} - pH_{min}) \cdot (pH_{max} - pH_{opt})}$
Zwietering *et al.*, 1996
$\mu_{max} = \mu_{opt} \cdot \frac{(pH - 4.72) \cdot (9.01 - pH)}{(6.48 - 4.72) \cdot (9.01 - 6.48)}$
3.0
2.5
2.0
1.5
1.0
0.5
0.0
μ_{max} (h^{-1})
4.0
5.0
6.0
7.0
8.0
pH
MSE=0.0075

γ ([HFo])

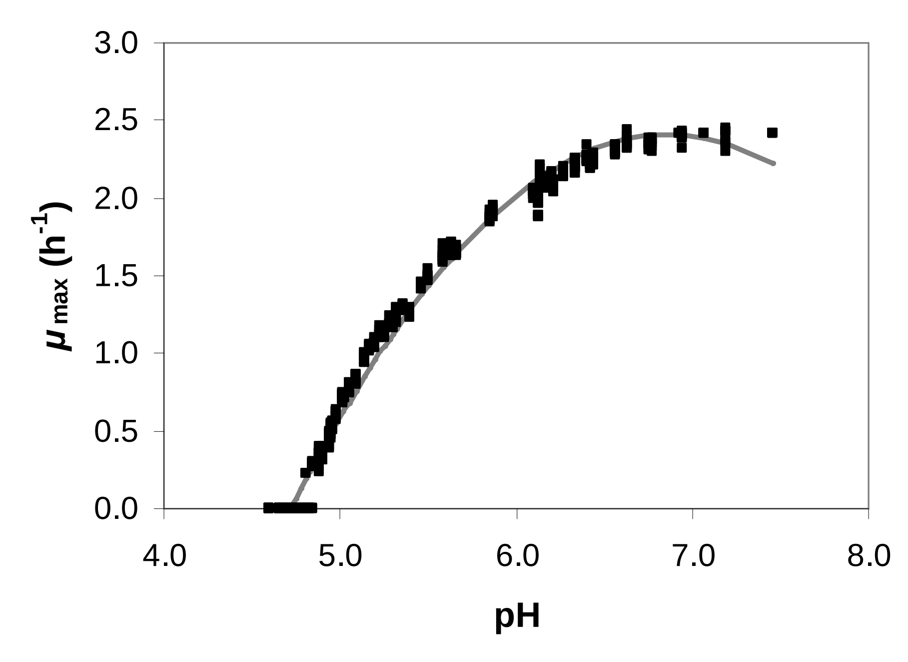


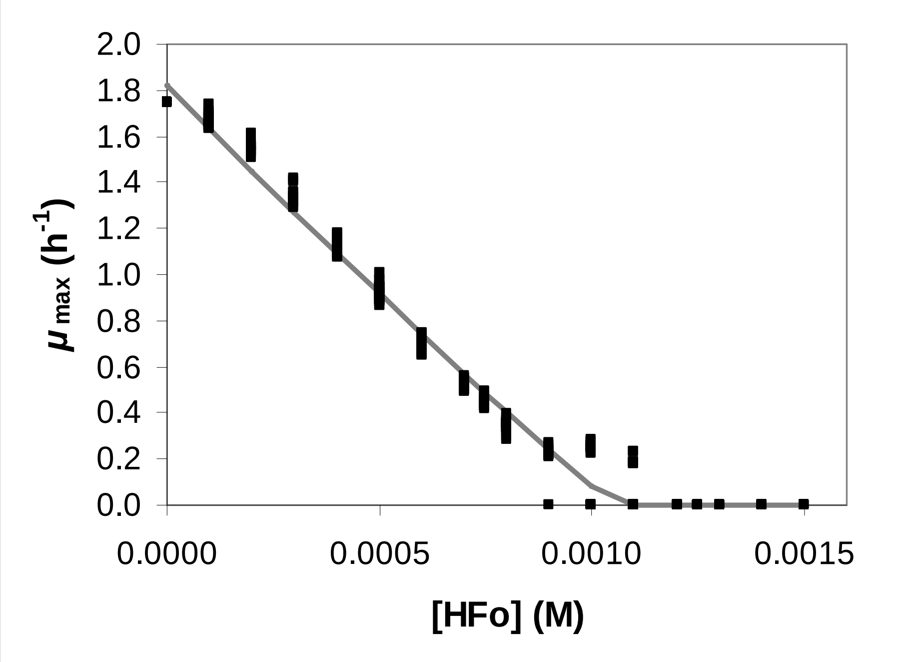
MSE=0.0042

Gamma hypothesis testing

pH effect $\gamma(pH) = \frac{(pH - 4.72)(9.01 - pH)}{(6.48 - 4.72)(9.01 - 6.48)}$

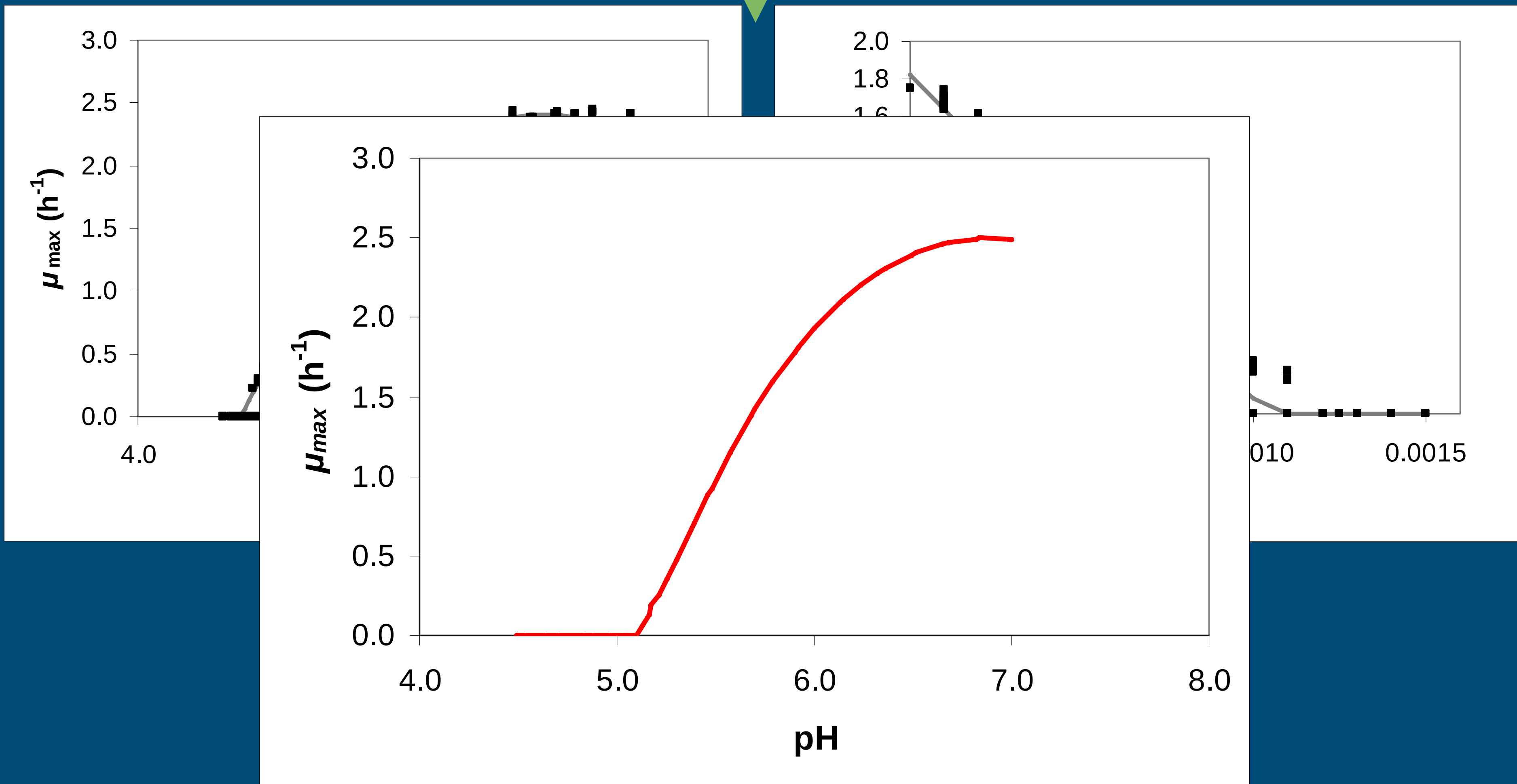
[HA] effect $\gamma([HA]) = \left(1 - \frac{[HA]}{0.0011}\right)^{1.08}$



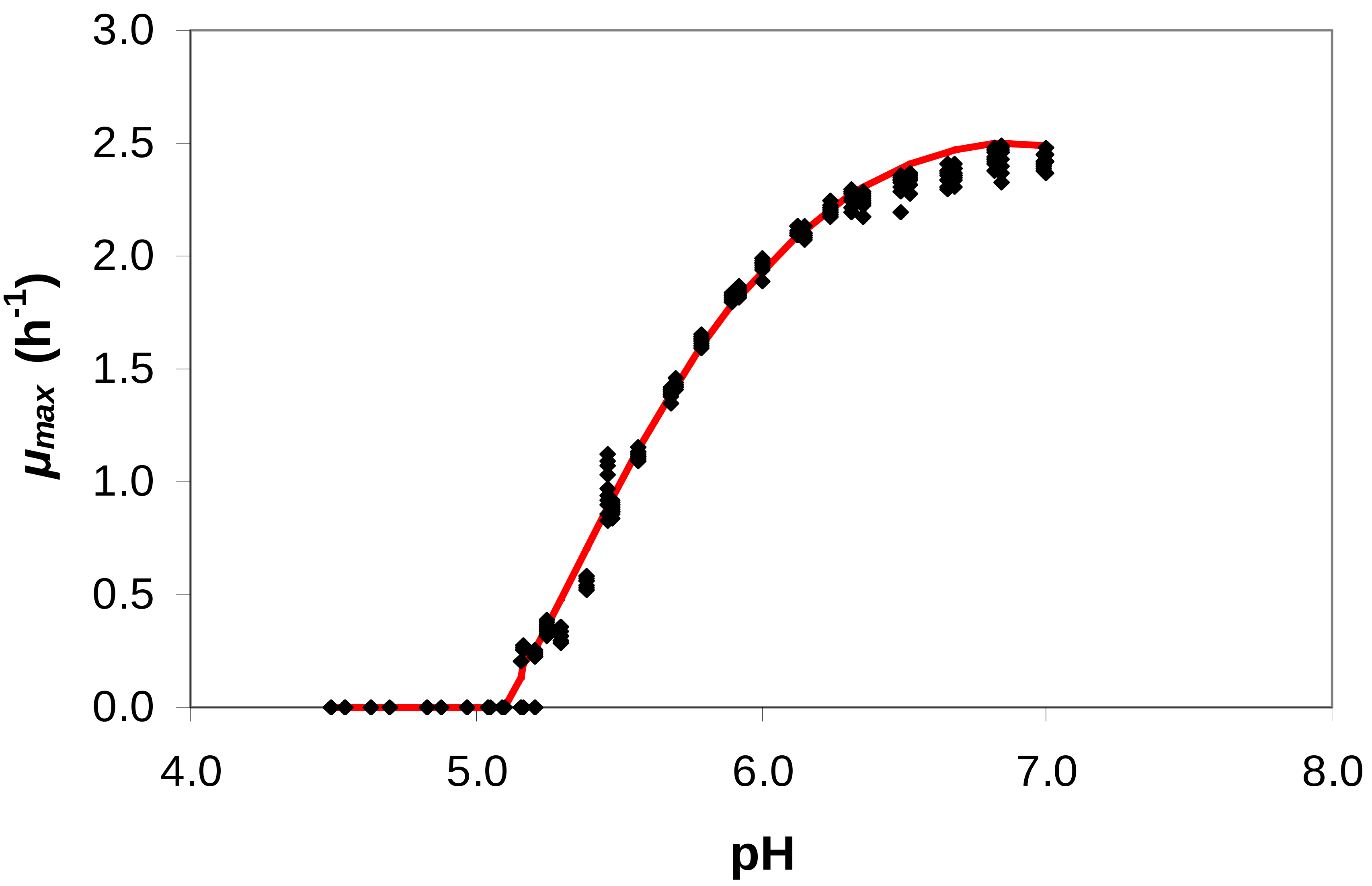


Gamma hypothesis testing

pH effect $\gamma(pH) = \frac{(pH - 4.72)(9.01 - pH)}{(6.48 - 4.72)(9.01 - 6.48)}$ * [HA] effect $\gamma([HA]) = \left(1 - \frac{[HA]}{0.0011}\right)^{1.08}$



Gamma hypothesis testing

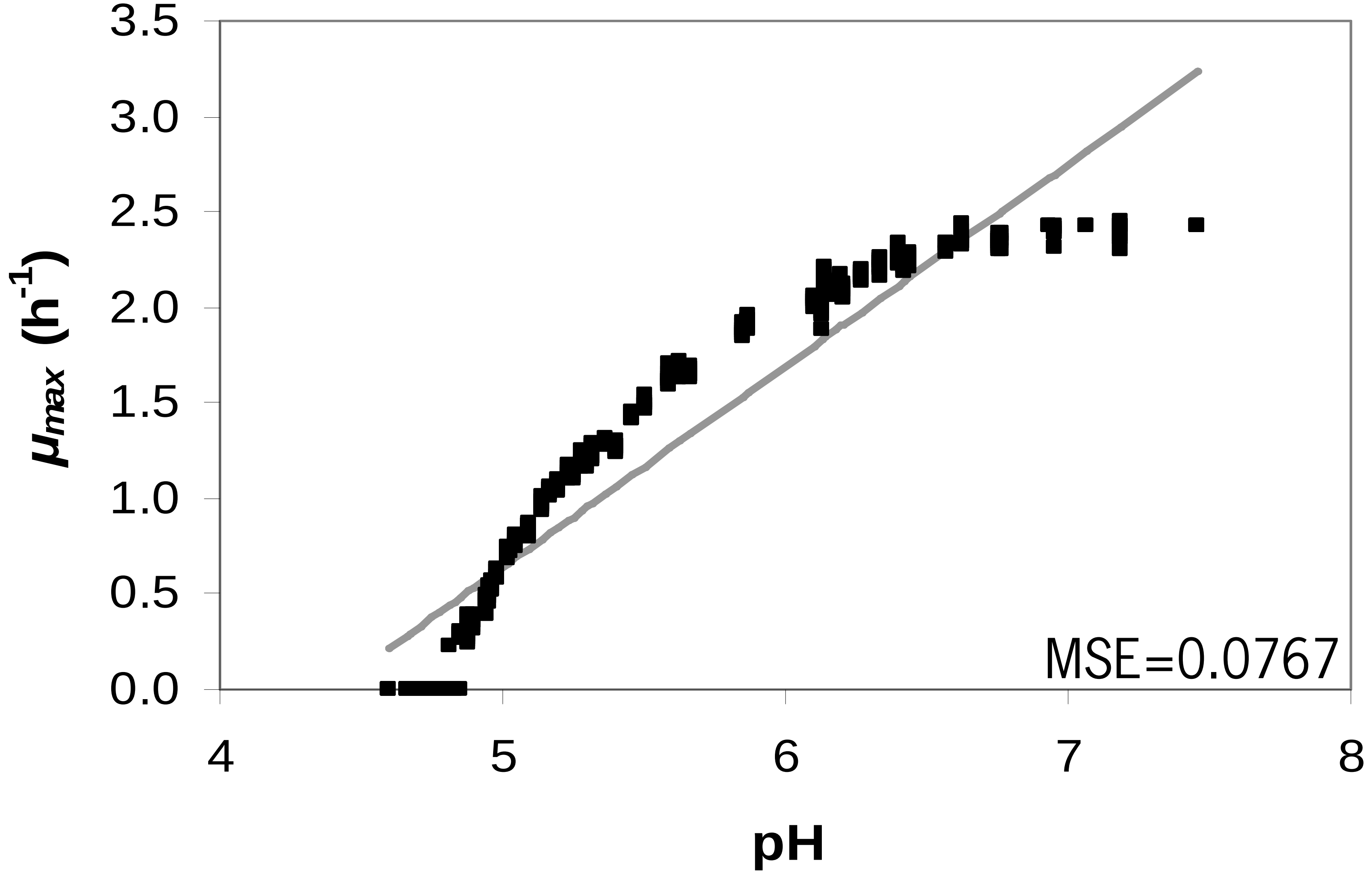


Goodness of fit PH models

Model	**reference**	**MSE**
$\mu_{\max} = \mu_{opt} \frac{(pH - pH_{\min})(pH_{\max} - pH)}{(pH_{opt} - pH_{\min})(pH_{\max} - pH_{opt})}$	Zwietering *et al.*, 1996	0.0075
$\sqrt{\mu_{\max}} = a\sqrt{pH - pH_{\min}}$	Ratkowsky *et al.*, 1982	
$\mu_{\max} = \mu_{opt}\left(1 - 10^{pH_{\min} - pH}\right)$	Presser *et al.*, 1997	
$\mu_{\max} = \mu_{opt}\left[1 - \frac{(pH - pH_{opt})^2}{(pH - pH_{opt})^2 + pH(pH_{\max} + pH_{\min} - pH) - pH_{\max} pH_{\min}}\right]$	Rosso *et al.*, 1995	

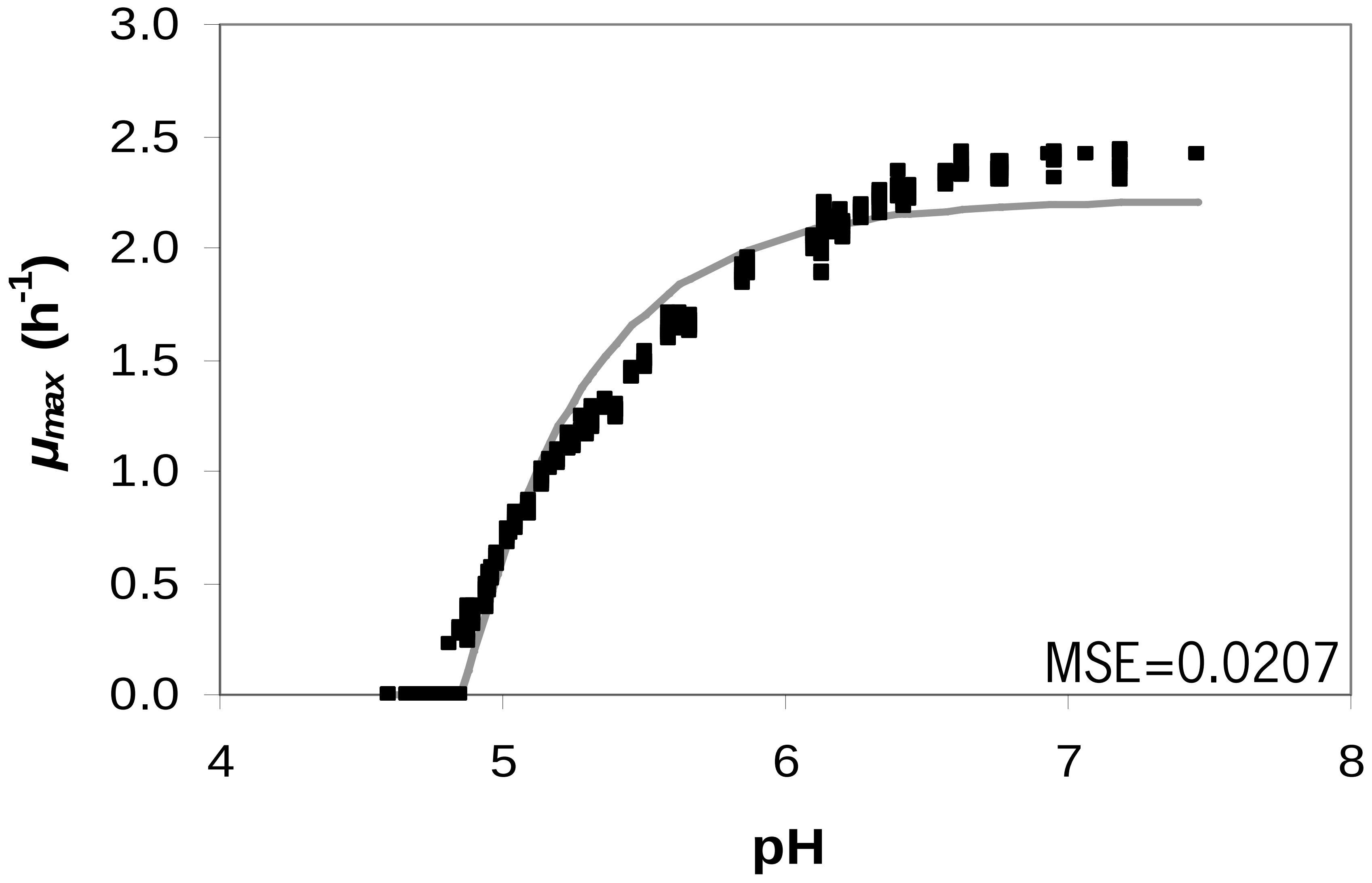
Model Ratkowsky *et al.*, 1982

$$\mu_{\max} = a^2 (pH - pH_{\min})$$



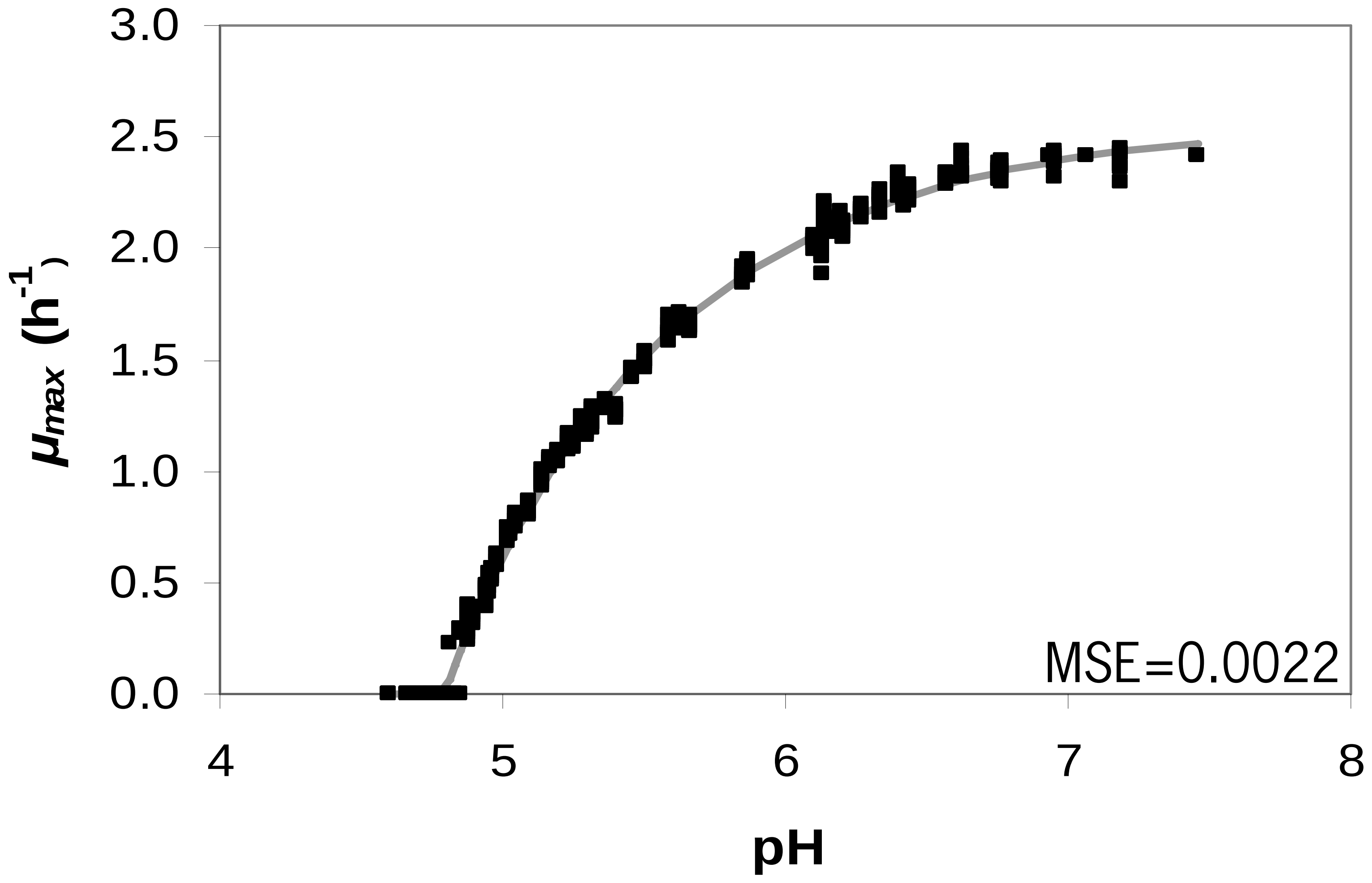
Model Presser *et al.*, 1993

$$\mu_{\max} = \mu_{opt}\left(1 - 10^{pH_{\min} - pH}\right)$$



Model Rosso *et al.*, 1994

$$\mu_{max} = \mu_{opt}\left[1 - \frac{(pH - pH_{opt})^2}{(pH - pH_{opt})^2 + pH(pH_{max} + pH_{min} - pH) - pH_{max}pH_{min}}\right]$$



Goodness of fit PH models

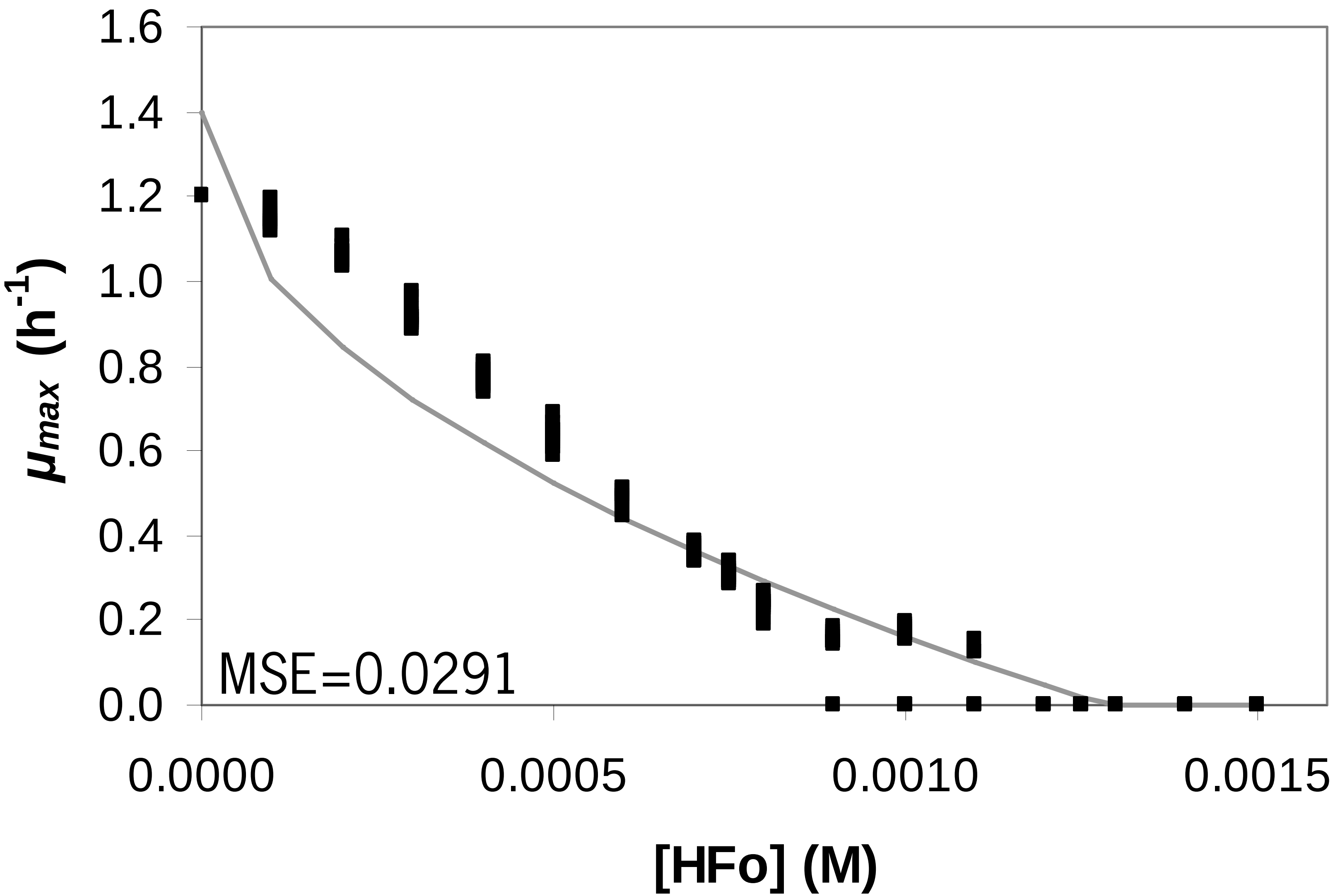
Model	reference	MSE
$\mu_{\max} = \mu_{opt} \dfrac{(pH - pH_{\min})(pH_{\max} - pH)}{(pH_{opt} - pH_{\min})(pH_{\max} - pH_{opt})}$	Zwietering *et al.*, 1996	0.0075
$\sqrt{\mu_{\max}} = a\sqrt{pH - pH_{\min}}$	Ratkowsky *et al.*, 1982	0.0767
$\mu_{\max} = \mu_{opt}\left(1 - 10^{pH_{\min} - pH}\right)$	Presser *et al.*, 1997	0.0207
$\mu_{\max} = \mu_{opt}\left[1 - \dfrac{\left(pH - pH_{opt}\right)^2}{\left(pH - pH_{opt}\right)^2 + pH\left(pH_{\max} + pH_{\min} - pH\right) - pH_{\max}pH_{\min}}\right]$	Rosso *et al.*, 1995	0.002

Goodness of fit [HA] models

Model	Reference	MSE
$\mu_{\max} = \mu_{opt}\left(1 - \frac{[Ha]}{[Ha]_{\max}}\right)^{\alpha}$	Pasos *et al.*, 1993	0.0042
$\mu_{\max} = \mu_{opt}\left(1 - \sqrt{\frac{[HA]}{[HA]_{\min}}}\right)$	Le Marc *et al.*, 2002	
$\mu_{\max} = \mu_{opt}\frac{a([HA]_{\min} - [HA])}{[HA]_{\min}(a - [HA])}$	Houtsma *et al.*, 1994	
$\mu_{\max} = \mu_{opt}\exp[-k([HA] - [HA]_{\min})]$	Yeh *et al.*, 1991	

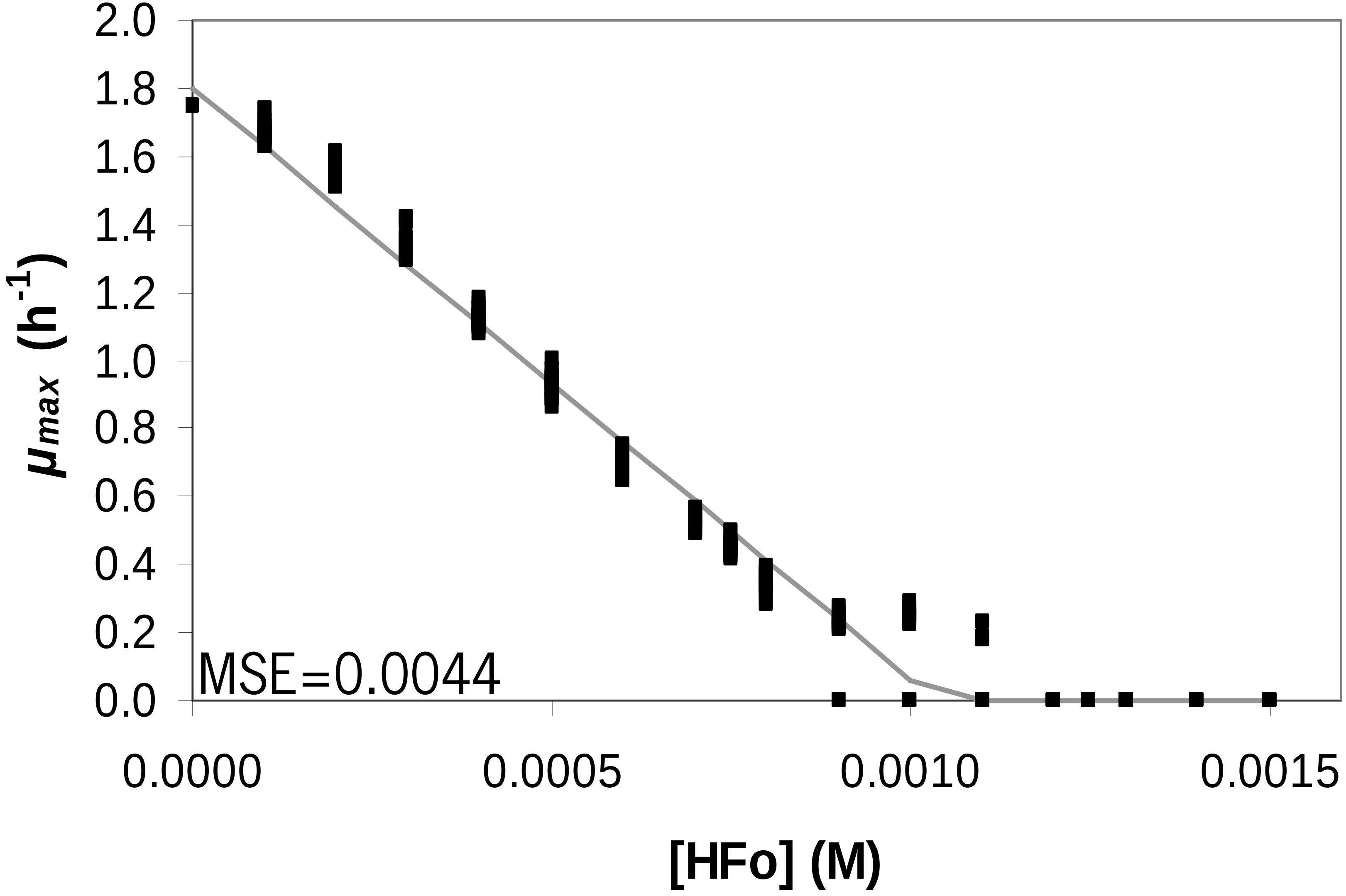
Model Le Marc *et al.*, 2002

$$\mu_{\max} = \mu_{opt}\left(1 - \sqrt{\frac{[HA]}{[HA]_{\min}}}\right)$$



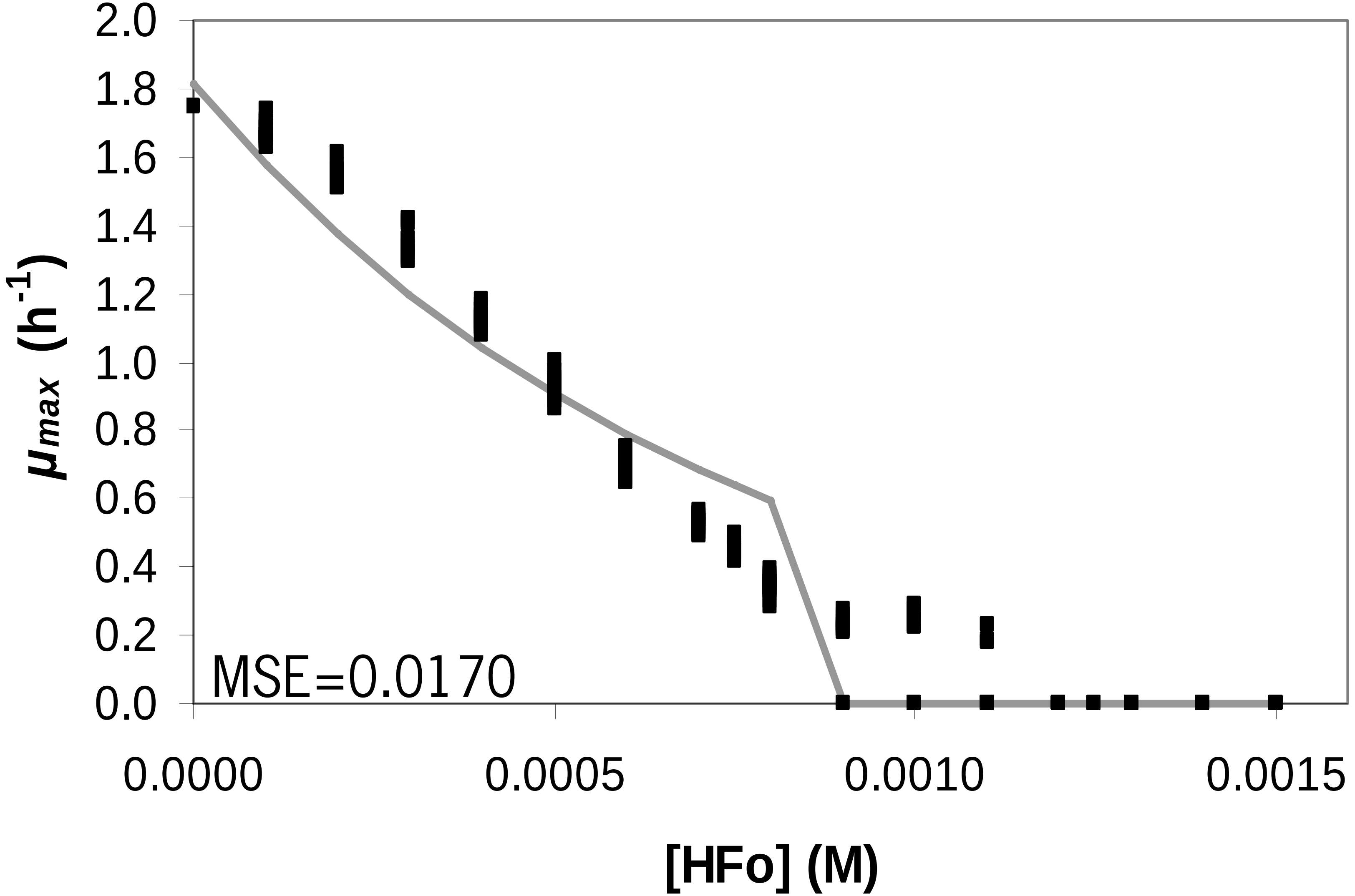
Model Houtsma *et al.*, 1994

$$\mu_{max} = \mu_{opt} \frac{a([HA]_{min} - [HA])}{[HA]_{min}(a - [HA])}$$



Model Yeh *et al.*, 1991

$$\mu_{max} = \mu_{opt} \exp[-k([HA]-[HA]_{min})]$$



Goodness of fit [HA] models

Model	Reference	MSE
$\mu_{\max} = \mu_{opt}\left(1 - \frac{[Ha]}{[Ha]_{\max}}\right)^{\alpha}$	Pasos *et al.*, 1993	0.0042
$\mu_{\max} = \mu_{opt}\left(1 - \sqrt{\frac{[HA]}{[HA]_{\min}}}\right)$	Le Marc *et al.*, 2002	0.0291
$\mu_{\max} = \mu_{opt}\frac{a([HA]_{\min} - [HA])}{[HA]_{\min}(a - [HA])}$	Houtsma *et al.*, 1994	0.0044
$\mu_{\max} = \mu_{opt}\exp[-k([HA] - [HA]_{\min})]$	Yeh *et al.*, 1991	0.0170

MSE of single and combined models

		$\mu_{max} = \mu_{opt}\left(1 - \frac{[HA]}{[HA]_{min}}\right)^{\alpha}$	$\mu_{max} = \mu_{opt}\left(1 - \sqrt{\frac{[HA]}{[HA]_{min}}}\right)$	$\mu_{max} = \mu_{opt}\frac{a([HA]_{min} - [HA])}{[HA]_{min}(a - [HA])}$	$\mu_{max} = \mu_{opt}\exp[-k([HA]-[HA]_{min})]$
		0.0042	0.0291	0.0044	0.0170
$\mu_{max} = \mu_{opt}\frac{(pH - pH_{min})(pH_{max} - pH)}{(pH_{opt} - pH_{min})(pH_{max} - pH_{opt})}$	0.0075				
$\mu_{max} = a^2(pH - pH_{min})$	0.0767				
$\mu_{max} = \mu_{opt}\left(1 - 10^{pH_{min} - pH}\right)$	0.0207				
$\mu_{max} = \mu_{opt}\left[1 - \frac{(pH - pH_{opt})^2}{(pH - pH_{opt})^2 + pH(pH_{max} + pH_{min} - pH) - pH_{max}pH_{min}}\right]$	0.0022				

MSE of single and combined models

		$\mu_{max}=\mu_{opt}\left(1-\frac{[HA]}{[HA]_{min}}\right)^{\alpha}$	$\mu_{max}=\mu_{opt}\left(1-\sqrt{\frac{[HA]}{[HA]_{min}}}\right)$	$\mu_{max}=\mu_{opt}\frac{a([HA]_{min}-[HA])}{[HA]_{min}(a-[HA])}$	$\mu_{max}=\mu_{opt}\exp[-k([HA]-[HA]_{min})]$
		0.0042	0.0291	0.0044	0.0170
$\mu_{max}=\mu_{opt}\frac{(pH-pH_{min})(pH_{max}-pH)}{(pH_{opt}-pH_{min})(pH_{max}-pH_{opt})}$	0.0075	0.0054	0.0749	0.0044	0.0095
$\mu_{max}=a^{2}(pH-pH_{min})$	0.0767	0.0401	0.0754	0.0399	0.0504
$\mu_{max}=\mu_{opt}\left(1-10^{pH_{min}-pH}\right)$	0.0207	0.0170	0.0861	0.0167	0.0210
$\mu_{max}=\mu_{opt}\left[1-\frac{(pH-pH_{opt})^{2}}{(pH-pH_{opt})^{2}+pH(pH_{max}+pH_{min}-pH)-pH_{max}pH_{min}}\right]$	0.0022	0.0062	0.0703	0.0057	0.0114

MSE of single and combined models

		$\mu_{max} = \mu_{opt}\left(1-\frac{[HA]}{[HA]_{min}}\right)^{\alpha}$	$\mu_{max} = \mu_{opt}\left(1-\sqrt{\frac{[HA]}{[HA]_{min}}}\right)$	$\mu_{max} = \mu_{opt}\frac{a([HA]_{min}-[HA])}{[HA]_{min}(a-[HA])}$	$\mu_{max} = \mu_{opt}\exp[-k([HA]-[HA]_{min})]$
		0.0042	0.0291	**0.0044**	0.0170
$\mu_{max} = \mu_{opt}\frac{(pH-pH_{min})(pH_{max}-pH)}{(pH_{opt}-pH_{min})(pH_{max}-pH_{opt})}$	**0.0075**	**0.0054**	0.0749	**0.0044**	0.0095
$\mu_{max} = a^2(pH-pH_{min})$	0.0767	0.0401	0.0754	0.0399	0.0504
$\mu_{max} = \mu_{opt}\left(1-10^{pH_{min}-pH}\right)$	0.0207	0.0170	0.0861	0.0167	0.0210
$\mu_{max} = \mu_{opt}\left[1-\frac{(pH-pH_{opt})^2}{(pH-pH_{opt})^2+pH(pH_{max}+pH_{min}-pH)-pH_{max}pH_{min}}\right]$	**0.0022**	**0.0062**	0.0703	**0.0057**	0.0114

$$\mu_{max,1b} = \mu_{opt,1b}\left(\frac{(pH-pH_{min,1b})(pH-pH_{max,1b})}{(pH-pH_{min,1b})(pH-pH_{max,1b})-(pH-pH_{opt,1b})^2}\right)\left(1-\sqrt{\frac{[HA]}{[HA]_{min}}}\right)\cdot\left((1-\gamma(pH))^2-(1-\tau([HA]))^2\right)$$

MSE=0.0692

Conclusions

- Selection of models for single hurdle required
- Parameters estimated based on single hurdles can describe combined effect
- Hurdles seem to combine in a multiplicative manner
- Preliminary results suggest that synergy factor does hardly reduce MSE between experimental data and model

Future work

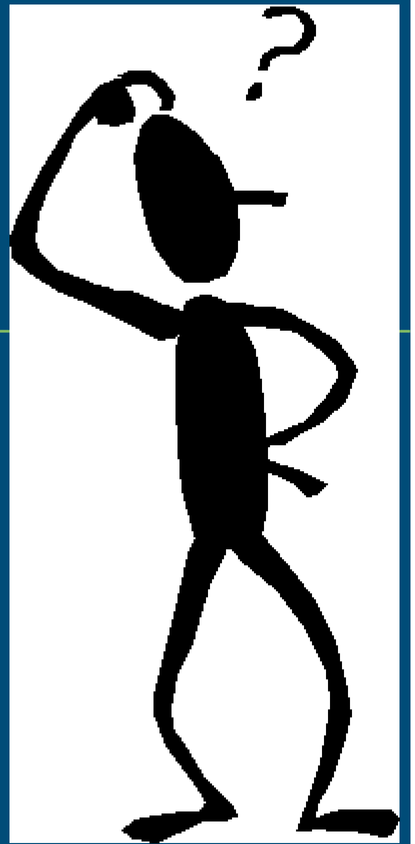
- Other acids
- More models (γ and γ+synergy)
- Test more quality parameters (F-test)
- Fit models to combined data sets and compare to γ model

Thank you

This research was financially supported by Nestec Ltd.

Thanks to: Han Joosten (Nestlé)

Martine Reij, Leon Gorris, Marcel Zwietering (WUR)



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