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ภาคผนวก

ภาคผนวก ก

สูตร และวิธีการเตรียมอาหารเลี้ยงเชื้อ

1. UVM broth : Selective primary enrichment medium

(1A) Base Medium¹

Proteose Peptone	5.0	g.
Tryptone	5.0	g.
Lab-Lamco'Powder	5.0	g.
Yeast extract	5.0	g.
Sodium chloride	20.0	g.
Di-sodium hydrogen phosphate	12.0	g.
Potassium dihydrogen phosphate	1.35	g.
Aesculin	1.0	g.
Water	1000	ml.

(1B) Listeria Primary Selective Enrichment Supplement (UVMI) SR0142B

Nalidaxic acid	45.0	mg.
Acriflavin	27.0	mg.
Sterile distilled water	10.0	ml.

อัตราส่วนการผสมอาหารเลี้ยงเชื้อ

(1A)Base Medium	2250	ml.
(1B) SR0142B	10.0	ml.

2. Fraser Broth : Selective secondary enrichment medium

(2A) Base Medium¹

Meat peptone	5.0	g.
Tryptone	5.0	g.
Meat extract	5.0	g.
Yeast extract	5.0	g.
Sodium chloride	20.0	g.
Disodium hydrogen phosphate dehydrate	12.0	g.
Potassium dihydrogen phosphate	1.35	g.
Aesculin	1.0	g.
Lithium chloride	3.0	g.
Sodium salt of nalidixic acid	0.02	g.
Water	1,000	ml.

(2B) Acriflavine hydrochloride solution²

Acriflavine hydrochloride	0.25	g.
Water	100	ml.

(2C) Ammonium iron(III) citrate solution²

Ammonium iron(III) citrate	5.0	g.
Water	100	ml.

อัตราส่วนการผสมอาหารเลี้ยงเชื้อ

(2A) Base Medium	10	ml.
(2B) Acriflavine hydrochloride solution	0.1	ml.
(2C) Ammonium iron(III) citrate solution	0.1	ml.

3. ALOA agar : First selective plating-out medium

(3A) Base medium¹

Enzymatic digest of animal tissues	18.0	g.
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Enzymatic digest of casein	6.0	g.
Yeast extract	10.0	g.
Sodium pyruvate	2.0	g.
Glucose	2.0	g.
Magnesium glycerophosphate	1.0	g.
Magnesium sulfate (anhydrous)	0.5	g
Sodium chloride	5.0	g
Lithium chloride	10.0	g.
Disodium hydrogen phosphate (anhydrous)	2.5	g.
5-Bromo-4-chloro-3-indolyl- β -D-glucopyranoside	0.05	g.
Agar	12 – 18	g.
Water	930	ml.
 (3B) Nalidixic acid solution		
Nalidixic acid sodium salt	0.02	g.
Sodium hydroxide (0.05 mol/l)	5	ml.
 (3C) Ceftazidime solution		
Ceftazidime	0.02	g.
Water	5	ml.
 (3D) Polymixin B solution		
Polymixin B sulfate	76 700	IU
Water	5	ml.
 (3E) Antibiotic supplement		
Cycloheximide solution		
Cyclohexamide	0.05	g.
HCl (1 mol/l)	2.5	ml.
Dimethylformamide (DMF)	7.5	ml.

Amphotericin B

Amphotericin B	0.01	g.
HCl (1 mol/l)	2.5	ml.
Dimethylformamide (DMF)	7.5	ml.

(3F) Supplement

L- α -phosphatidylinsital (Sigma P6636 ^{®3})	2.0	g.
Cold water	50	ml.

อัตราส่วนการผสมอาหารเลี้ยงเชื้อ

(3A) Base medium	930	ml.
(3B) Nalidixic acid solution	5.0	ml.
(3C) Ceftazidime solution	5.0	ml.
(3D) Polymixin B solution	5.0	ml.
(3E) Cycloheximide solution	5.0	ml.
Or Amphotericin B solution	10.0	ml.
(3F) Supplement	50.0	ml.

4. Oxford agar : Second selective plating-out medium

(4A) Agar base^①

Proteose peptone	23.0	g.
Starch	1.0	g.
Sodium chloride	5.0	g.
Aesculin	1.0	g.
Lithium chloride	15.0	g.
Ammonium iron(III) citrate	0.5	g.
Agar	9-18	g.
Water	1,000	ml.

(4B) Supplement for 1,000 ml. Medium²

Cycloheximide	400	mg.
Colistin sulfate	20	mg.
Acriflavine hydrochloride	5.0	mg.
Cefotetan	2.0	mg.
Fosfomycin	10	mg.
Ethanol	5.0	ml.
Water	5.0	ml.

5. Tryptone soya yeast extract agar (TSYEA) : Solid culture medium¹

Tryptone	17.0	g.
Soya peptone	3.0	g.
Sodium chloride	5.0	g.
Dipotassium phosphate	2.5	g.
Glucose	2.5	g.
Yeast extract	6.0	g.
Agar	9-18	g.
Water	1,000	ml.

6. Sheep blood agar

(6A) Base medium¹

Meat peptone	15	g.
Liver digest	2.5	g.
Yeast extract	5.0	g.
Sodium chloride	5.0	g.
Agar	9-18	g.
Water	1,000	ml.
Defibrinate sheep blood	50-70	ml.

7. Carbohydrate utilization broth Rhamnose broth

(7A) Base medium¹

Proteose peptone	1.0	g.
Meat extract	1.0	g.
Sodium chloride	5.0	g.
Bromocresol purple	0.02	g.
Water	1,000	ml.

(7B) Rhamnose solution²

Rhamnose	5.0	g.
Water	100	ml.

(7C) Xylose solution²

Xylose	5.0	g.
Water	100	ml.

อัตราส่วนการผสมอาหารเลี้ยงเชื้อ

(7A) Base medium	9.0	ml.
(7B) หรือ (7C)	1.0	ml.

8. Motility agar¹

Casein peptone	20.0	g.
Meat peptone	6.1	g.
Agar	3.5	g.
Water	1,000	ml.

9. CAMP medium

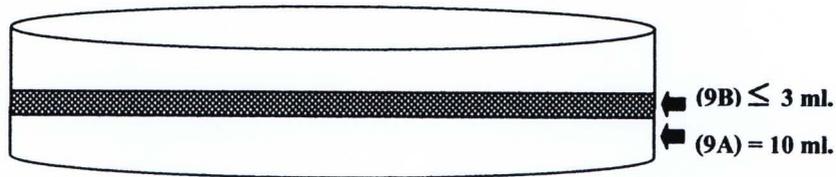
(9A) Base medium¹

Meat peptone	15	g.
Liver digest	2.5	g.

Yeast extract	5.0	g.
Sodium chloride	5.0	g.
Agar	9-18	g.
Water	1,000	ml.

(9B) Sheep blood medium ตามข้อ (6)

เทอาหารเลี้ยงเชื้อข้อ (9A) ใส่เพลทๆละ 10 ml. ทิ้งไว้ให้แข็งตัวในอุณหภูมิห้องประมาณ 15 นาทีแล้วเททับด้วย (9B) โดยปริมาตรไม่เกิน 3 ml. ต่อเพลท หากมีการเตรียมอาหารเลี้ยงเชื้อ (9A) ไว้ล่วงหน้าให้นำออกมาทิ้งไว้ที่อุณหภูมิ 37 °C ประมาณ 20 นาที ก่อนแล้วจึงเททับด้วย (9B)



รูปที่ 20: การเทเพลท CAMP test medium

หมายเหตุ

- ① Dissolve the components in water. Sterilize for 15 min in the autoclave set at 121°C
- ② Sterilize by filtration

*** After sterilization is pH 7.2±0.2 at 25°C

ภาคผนวก ข

1. ข้อมูลค่าความสัมพันธ์ค่าปริมาณน้ำใช้ได้ (water activity) ในเนื้อไก่ปรุงสุก ของแต่ละร้อยละ ความเข้มข้นของเกลือโซเดียมคลอไรด์ (NaCl)

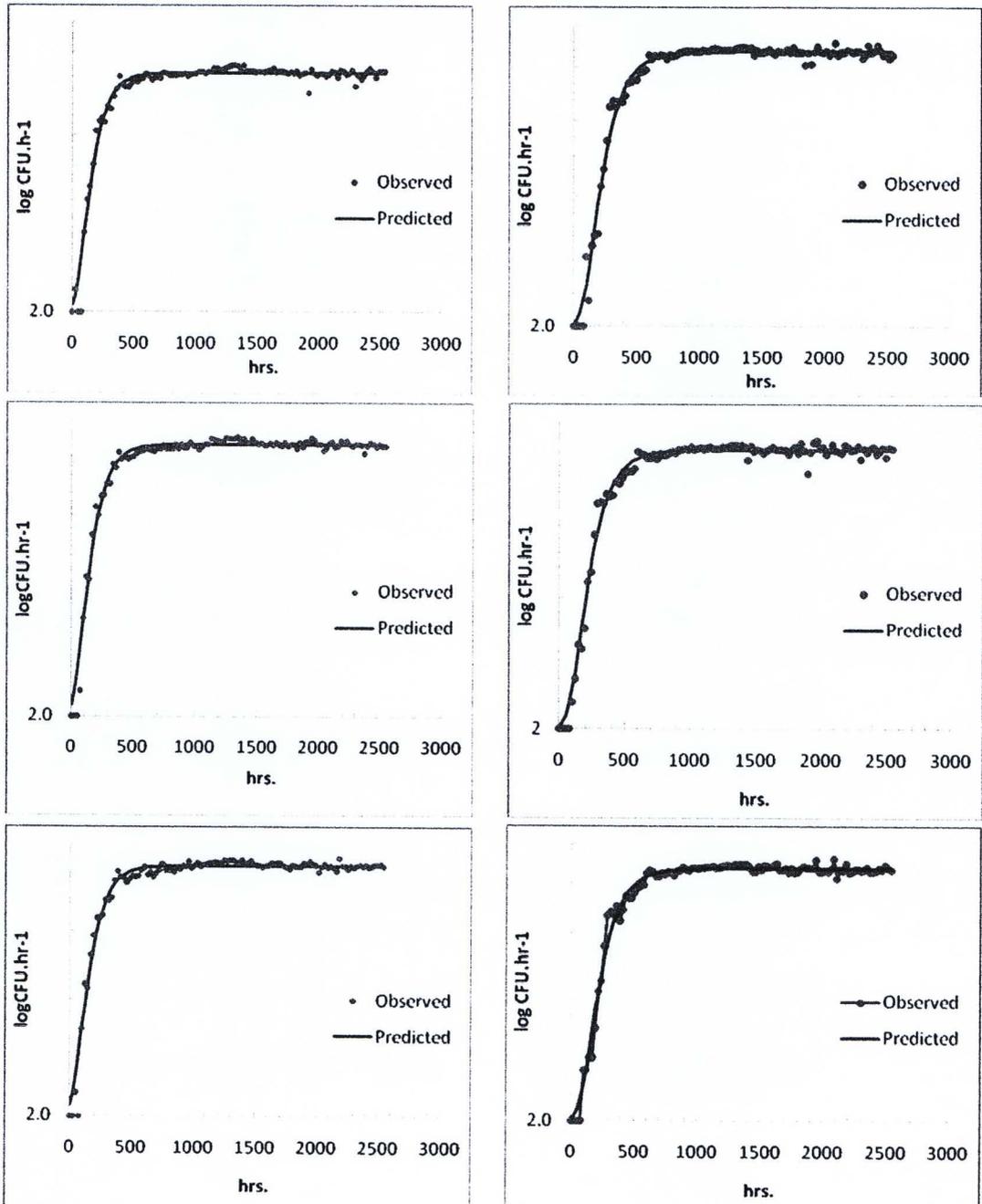
%NaCl (w/w)	ค่าปริมาณน้ำใช้ได้ (water activity, a_w)*				
	2	4	6	8	10
1	0.9800	0.9660	0.9440	0.9510	0.9270
2	0.9760	0.9740	0.9560	0.9310	0.9200
3	0.9750	0.9630	0.9440	0.9340	0.8950
4	0.9820	0.9680	0.9540	0.9580	0.9240
5	0.9800	0.9590	0.9340	0.9530	0.9230
6	0.9820	0.9760	0.9510	0.9430	0.9060
7	0.9810	0.9720	0.9470	0.9490	0.9100
8	0.9780	0.9670	0.9510	0.9400	0.9410
9	0.9800	0.9620	0.9610	0.9590	0.9350
10	0.9760	0.9730	0.9600	0.9570	0.9350
ค่าเฉลี่ย	0.9790	0.9680	0.9502	0.9475	0.9216

2. เส้นโค้งการเจริญของ *L.monocytogenes* แต่ละคู่ปัจจัย ที่ทดสอบความเหมาะสมของข้อมูล การเจริญตามสมการของ Gompertz

2.1 ที่อุณหภูมิ 5 องศาเซลเซียส

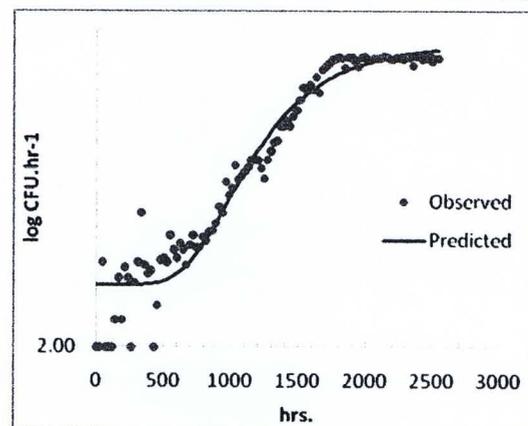
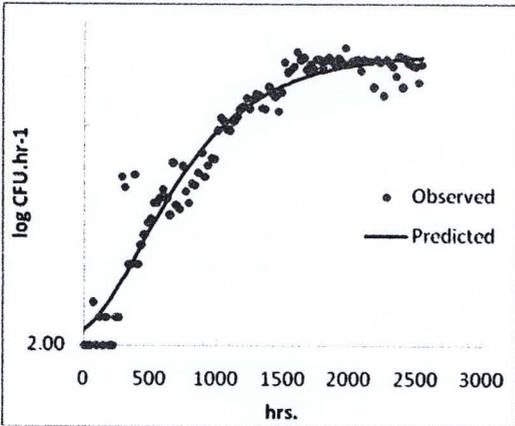
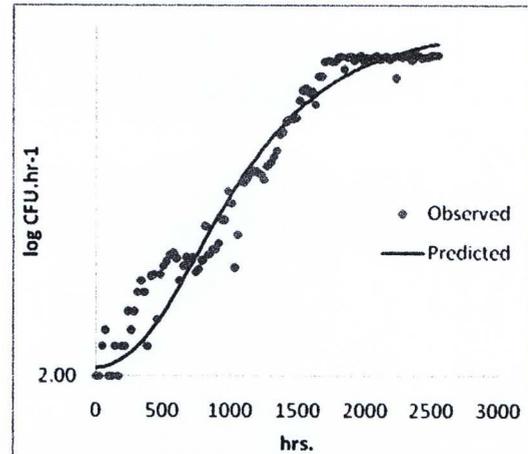
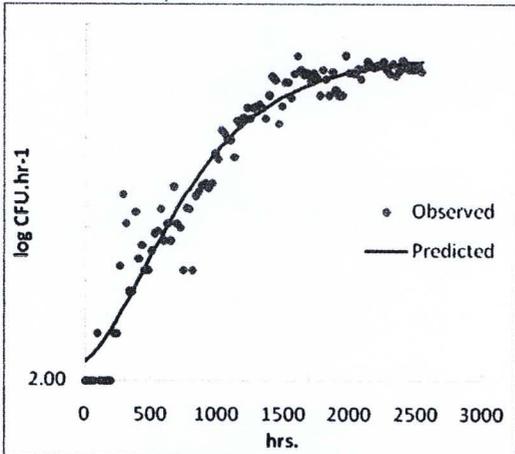
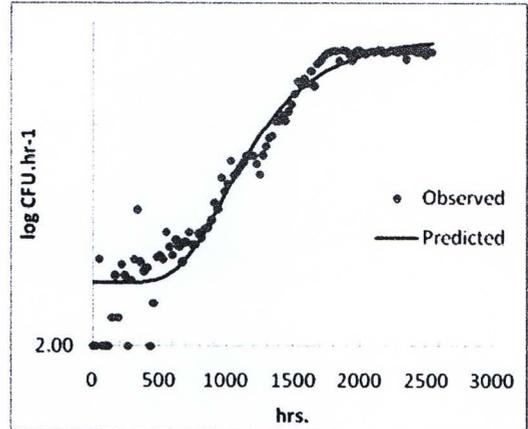
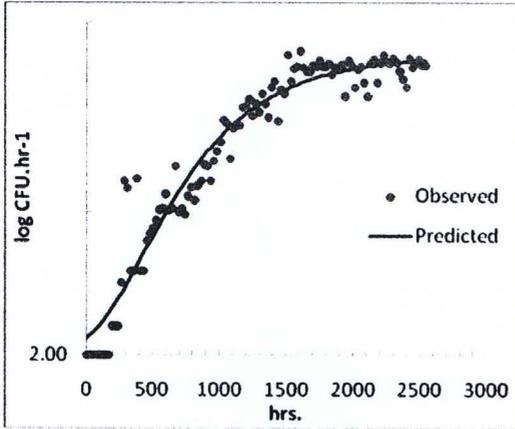
(1) 2%NaCl

(2) 4%NaCl



(3) 6%NaCl

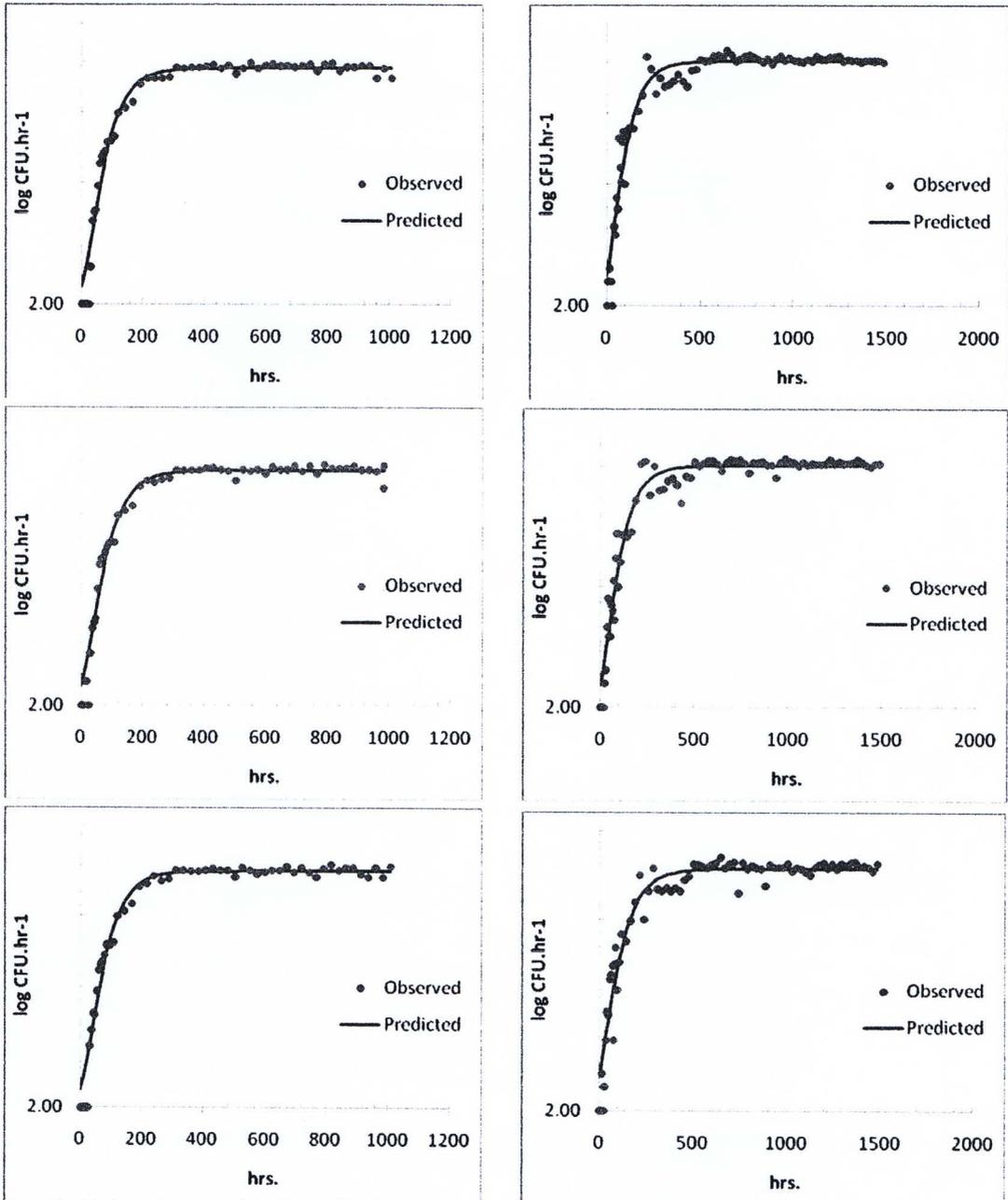
(4) 8%NaCl



2.2 ที่อุณหภูมิ 10 องศาเซลเซียส

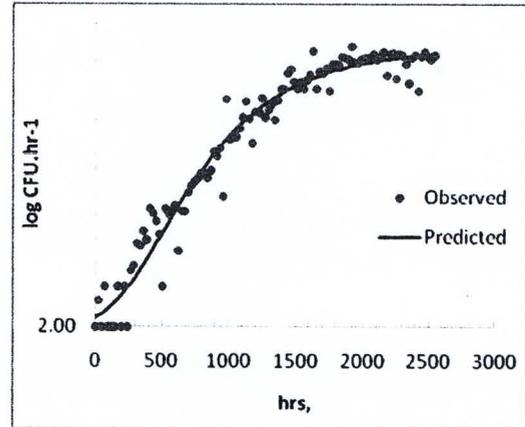
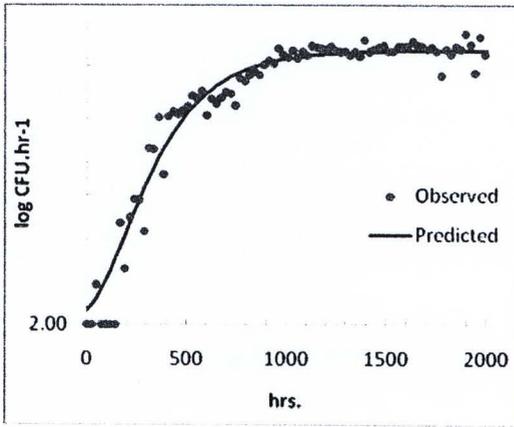
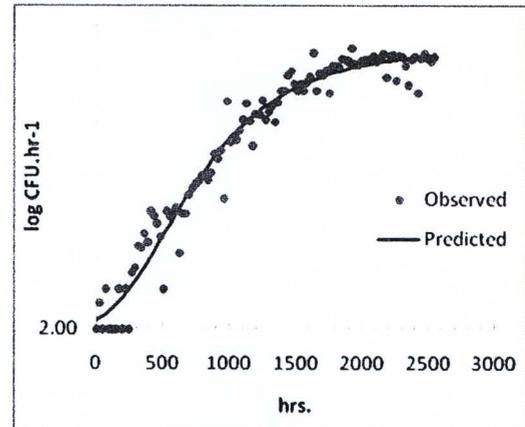
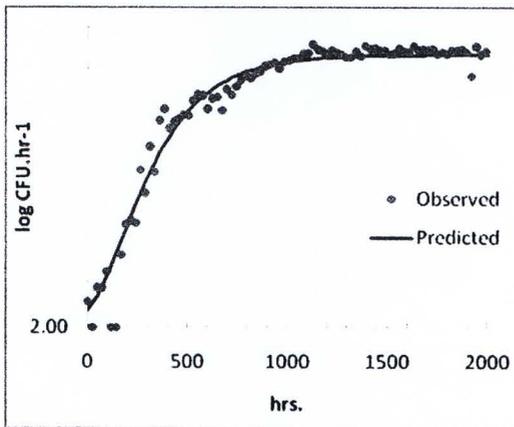
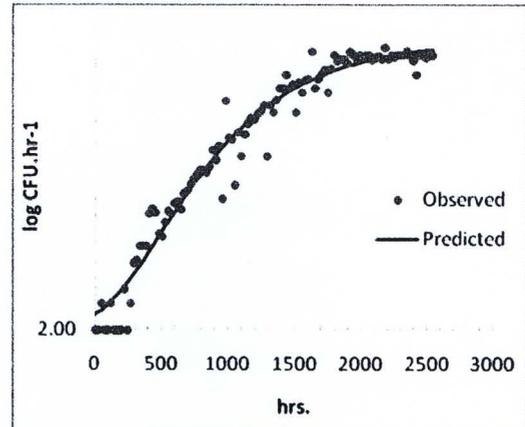
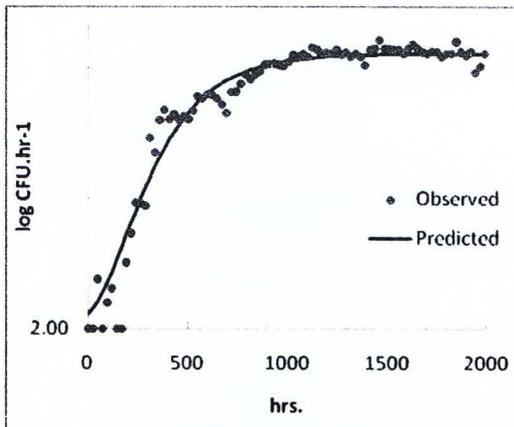
(1) 2%NaCl

(2) 4%NaCl



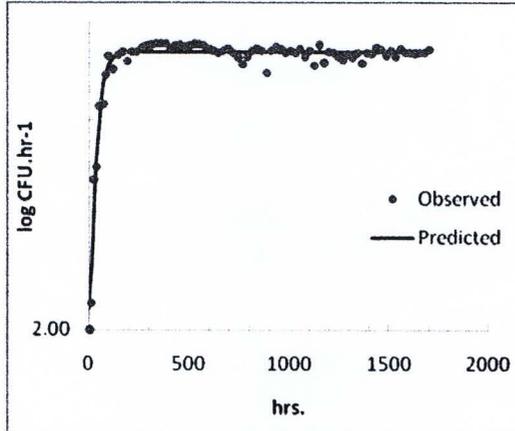
(3) 6%NaCl

(4) 8%NaCl

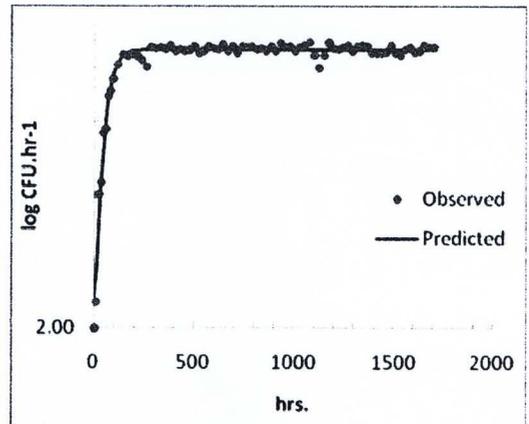
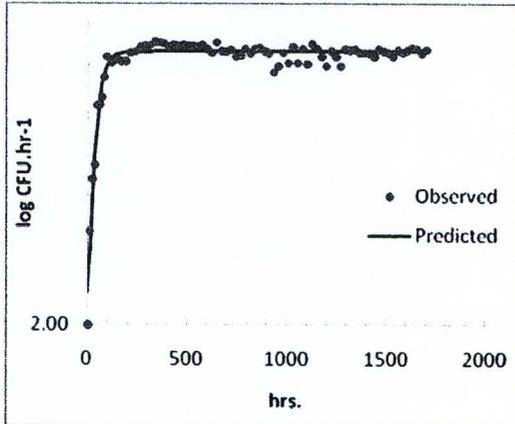
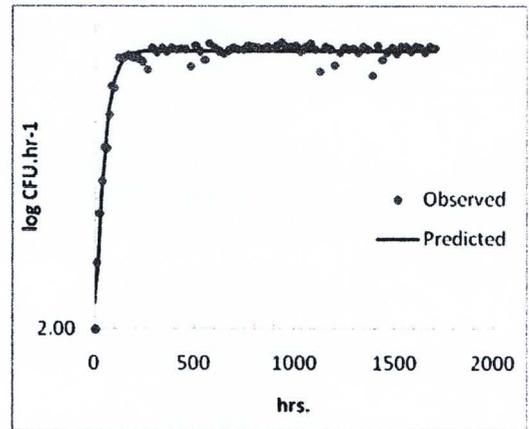
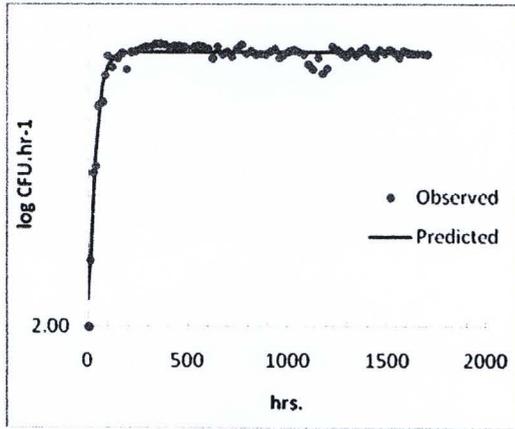
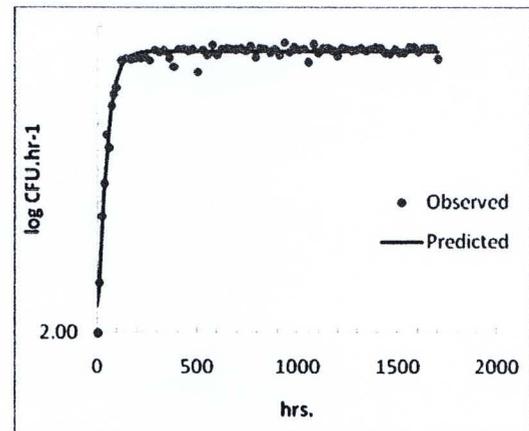


2.3 ที่อุณหภูมิ 15 องศาเซลเซียส

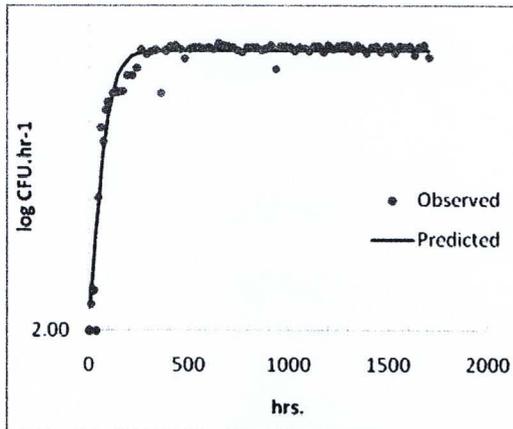
(1) 2%NaCl



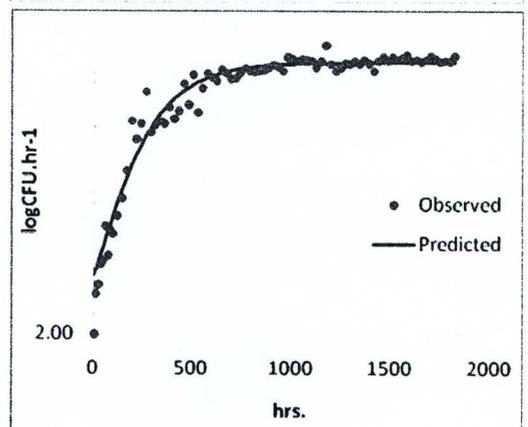
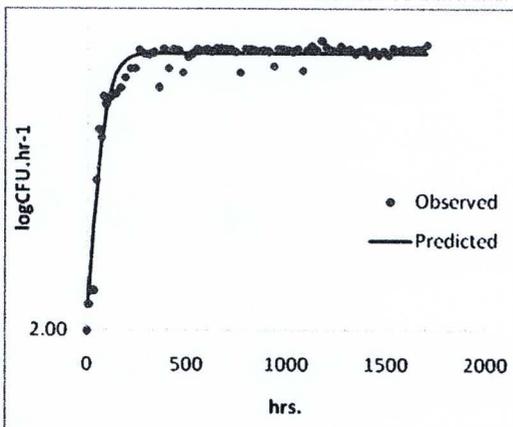
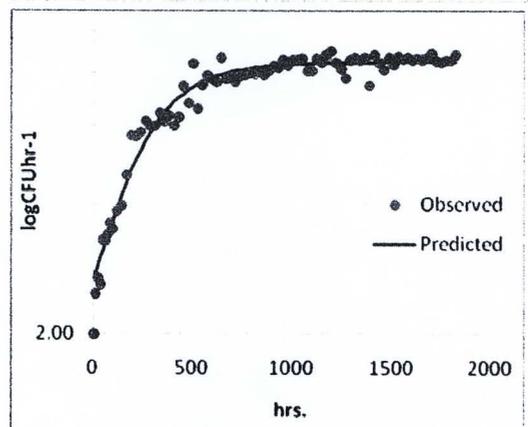
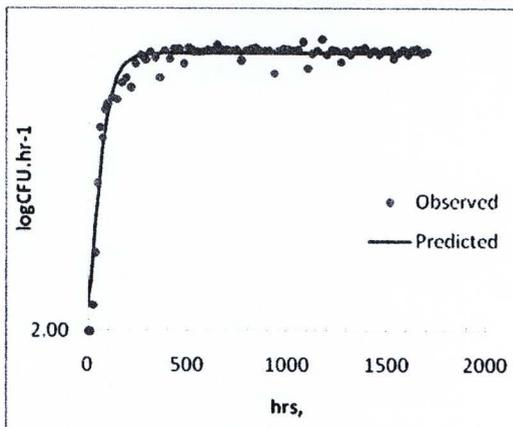
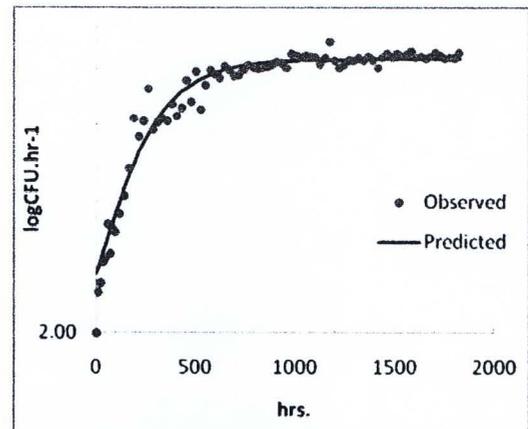
(2) 4%NaCl



(3) 6%NaCl



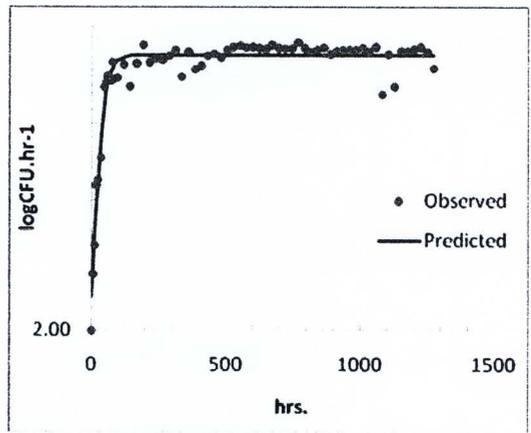
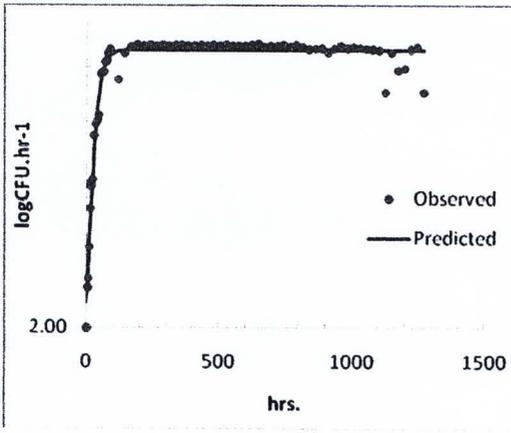
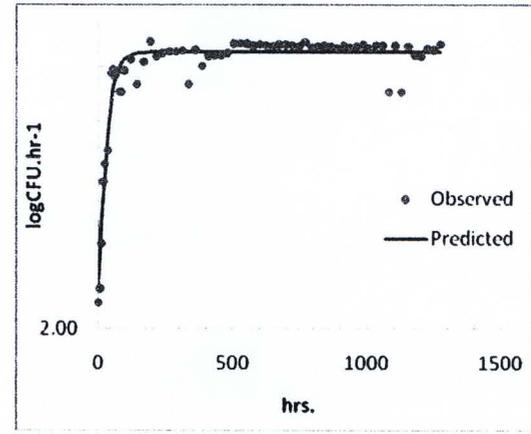
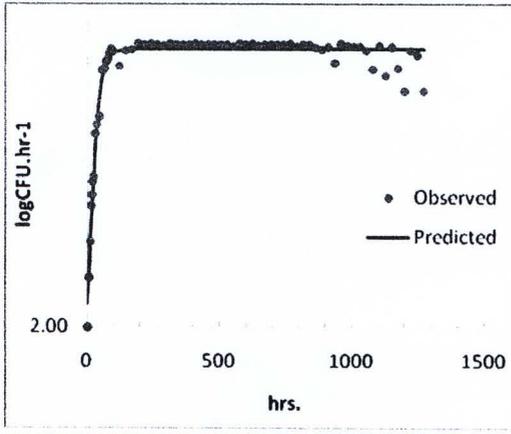
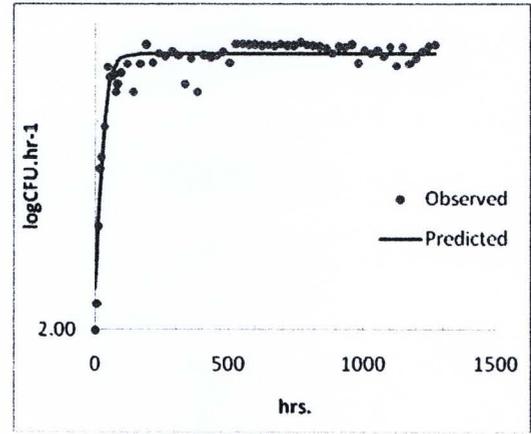
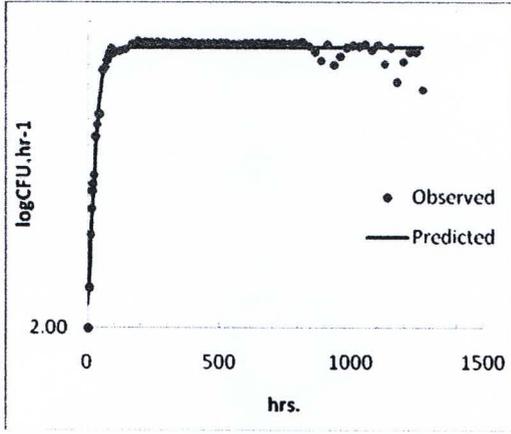
(4) 8%NaCl



2.4 ที่อุณหภูมิ 20 องศาเซลเซียส

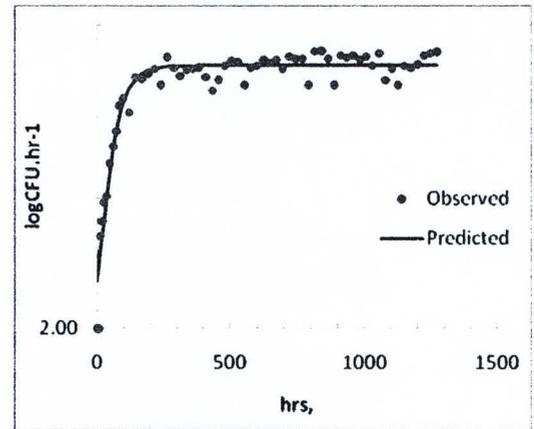
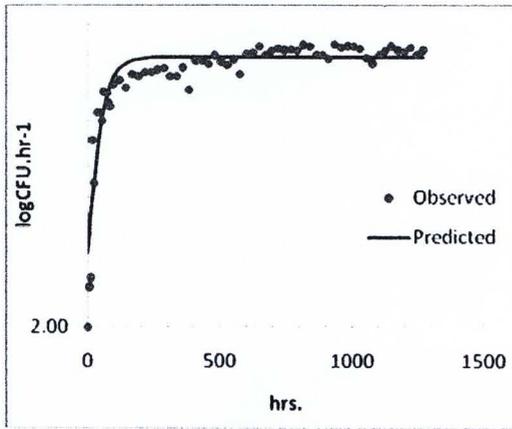
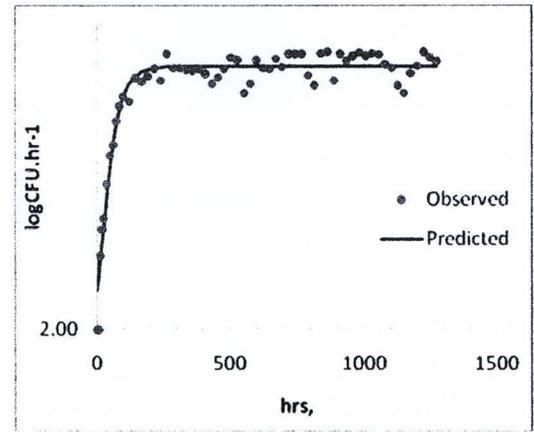
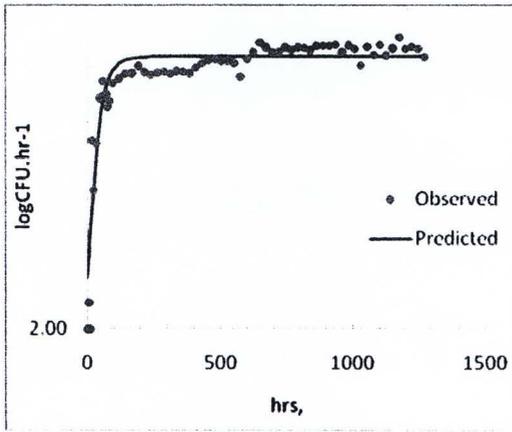
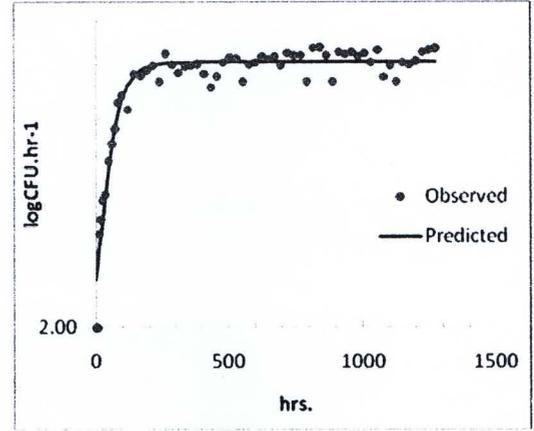
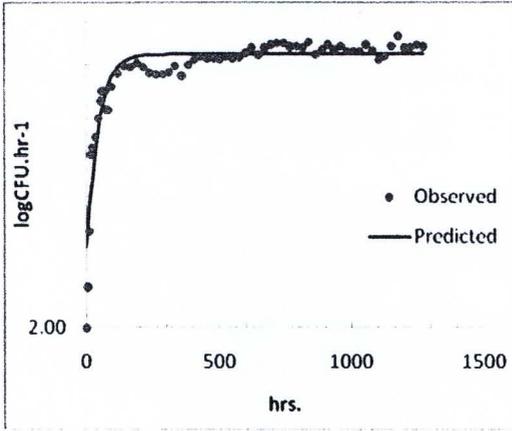
(1) 2%NaCl

(2) 4%NaCl



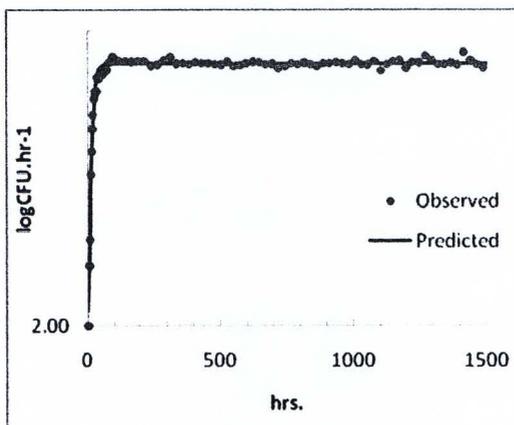
(3) 6%NaCl

(4) 8%NaCl

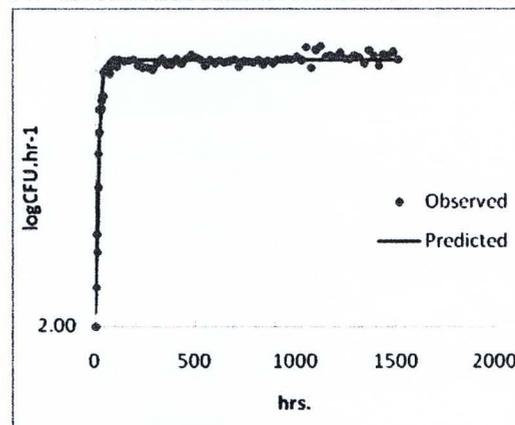
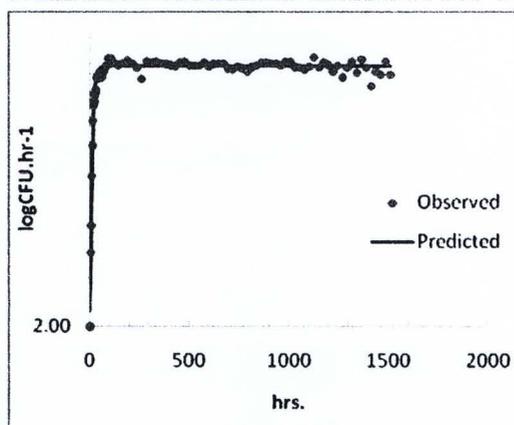
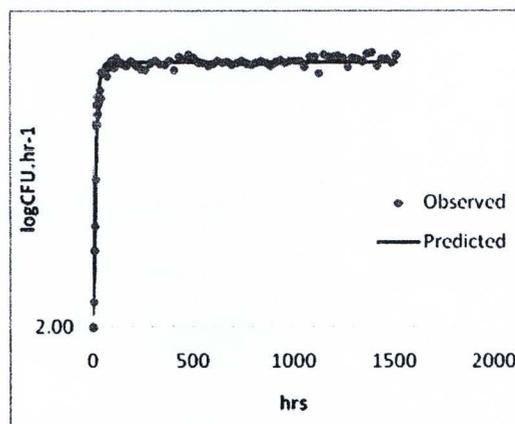
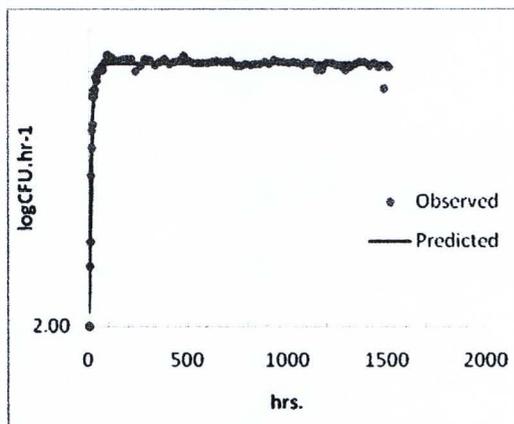
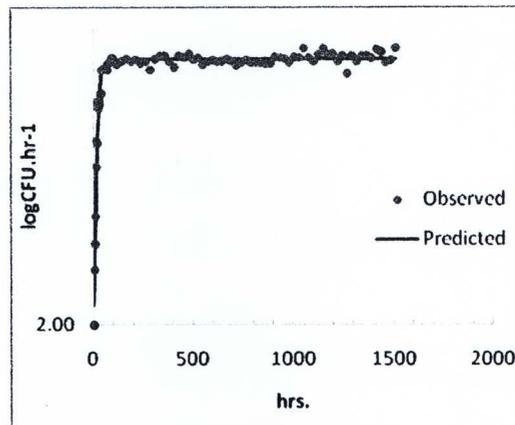


2.5 ที่อุณหภูมิ 25 องศาเซลเซียส

(1) 2%NaCl

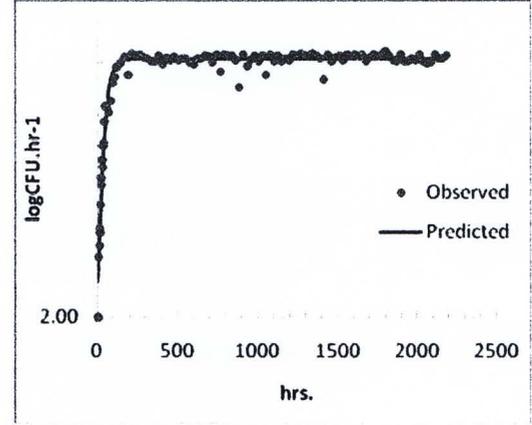
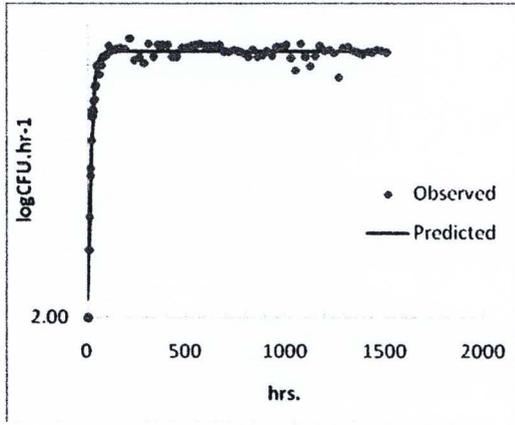
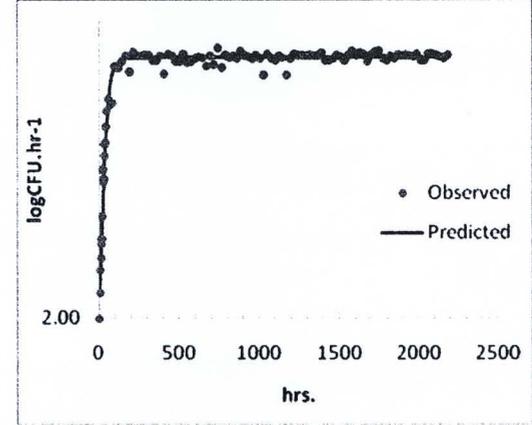
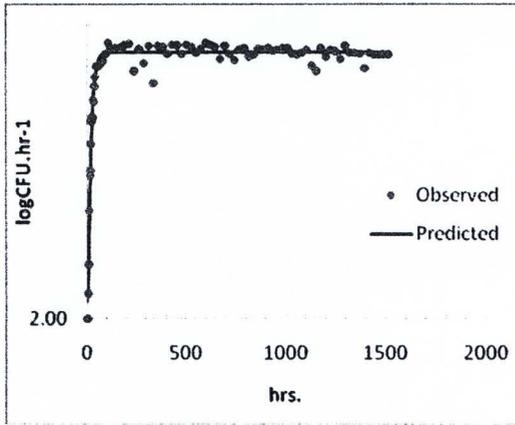
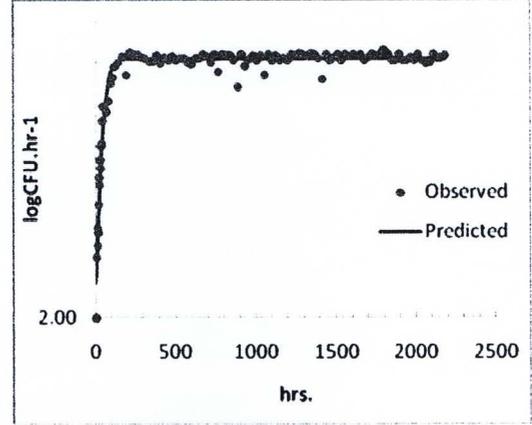
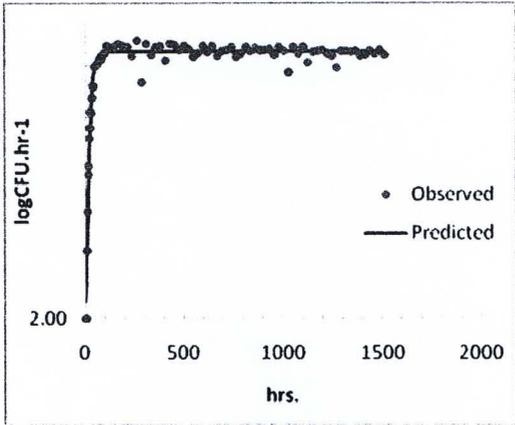


(2) 4%NaCl



(3) 6%NaCl

(4)8%NaCl



ภาคผนวก ค

1. ค่าการทำนายอัตราการเจริญเติบโตของ *L.monocytogenes* ในเนื้อไก่ปรุงสุก โดยแบบจำลองคณิตศาสตร์ สำหรับที่อุณหภูมิระหว่าง 10-25 องศาเซลเซียส และปริมาณน้ำใช้ได้ระหว่าง 0.979-0.950

$$\sqrt{r} = 0.061 \cdot \sqrt{a_w - 0.902} \cdot (T - 272.4)$$

a _w	Temp. (°C)	Growth rate (cfu.h ⁻¹)		%diff
		Predicted	observed	
0.979	25	0.192	0.245	21.537%
	20	0.125	0.121	3.300%
	15	0.072	0.088	18.039%
	10	0.034	0.038	11.288%
0.968	25	0.165	0.201	17.698%
	20	0.107	0.107	0.085%
	15	0.062	0.072	13.636%
	10	0.029	0.027	5.241%
0.950	25	0.121	0.171	29.27%
	20	0.079	0.067	18.312%
	15	0.045	0.051	10.819%
	10	0.021	0.010	104.015%

โดยมีค่า Bias factor	เท่ากับ	1.033
Accuracy factor	เท่ากับ	1.213
RMSE	เท่ากับ	0.024

2. ค่าการทำนายอัตราการเจริญเติบโตของ *L.monocytogenes* ในเนื้อไก่ปรุงสุก โดยแบบจำลองคณิตศาสตร์ $\sqrt{r} = \beta\sqrt{a_w - a_{wmin}} \cdot (T - T_{min})$ สำหรับที่อุณหภูมิระหว่าง 10-25 องศาเซลเซียส และปริมาณน้ำใช้ได้ระหว่าง 0.979-0.950 ซึ่งใช้ค่า T_{min} และ a_{wmin} จำเพาะแต่ละคู่ของปัจจัย

β	T_{min}	a_{wmin}	a_w	Temp. (°C)	Growth rate (cfu.h ⁻¹)		%diff
					Predicted	observed	
0.061	270.4	0.903	0.979	25	0.217	0.245	11.21%
				20	0.146	0.121	20.98%
				15	0.089	0.088	1.42%
				10	0.046	0.038	21.59%
				5	0.017	0.023	28.24%
	271.0	0.901	0.968	25	0.183	0.201	8.50%
				20	0.122	0.107	13.85%
				15	0.073	0.072	2.41%
				10	0.036	0.027	34.43%
				5	0.012	0.018	31.31%
	272.8	0.898	0.950	25	0.124	0.171	27.02%
				20	0.080	0.067	20.73%
				15	0.045	0.051	10.12%
				10	0.020	0.010	100.64%
				5	0.005	0.004	22.78%
	275.5	0.906	0.947	25	0.079	0.085	7.26%
				20	0.048	0.057	16.13%
				15	0.024	0.012	105.07%
				10	0.009	0.004	101.40%
				5	0.001	0.005	78.16%

โดยมีค่า Bias factor	เท่ากับ	0.987
Accuracy factor	เท่ากับ	1.381
RMSE	เท่ากับ	0.015

3. ค่าการทำนายอัตราการเจริญเติบโตของ *L.monocytogenes* ในเนื้อไก่ปรุงสุก โดยแบบจำลองคณิตศาสตร์ $\sqrt{r} = \beta\sqrt{a_w - a_{wmin}} \cdot (T - T_{min})$ สำหรับที่อุณหภูมิระหว่าง 10-25 องศาเซลเซียส และปริมาณน้ำใช้ได้ระหว่าง 0.979-0.950 ซึ่งใช้ค่า T_{min} ที่จำเพาะแต่ละคู่ของปัจจัย

β	T_{min}	a_{wmin}	a_w	Temp. (°C)	Growth rate (cfu.h ⁻¹)		%diff
					Predicted*	observed	
0.061	270.4	0.902	0.979	25	0.220	0.245	10.04%
				20	0.148	0.121	22.58%
				15	0.090	0.088	2.76%
				10	0.046	0.038	23.19%
				5	0.017	0.023	27.30%
	271.0	0.902	0.968	25	0.181	0.201	9.87%
				20	0.120	0.107	12.15%
				15	0.072	0.072	0.88%
				10	0.036	0.027	32.43%
				5	0.012	0.018	32.34%
	272.8	0.902	0.950	25	0.115	0.171	32.61%
				20	0.074	0.067	11.48%
				15	0.042	0.051	17.01%
				10	0.019	0.010	85.27%
				5	0.005	0.004	13.37%
	275.5	0.902	0.947	25	0.086	0.085	1.67%
				20	0.052	0.057	8.05%
				15	0.027	0.012	124.84%
				10	0.009	0.004	120.82%
				5	0.001	0.005	76.06%

โดยมีค่า Bias factor	เท่ากับ	0.985
Accuracy factor	เท่ากับ	1.372
RMSE	เท่ากับ	0.016



ประวัติผู้เขียนงานวิจัย

นางสาวเสาวลักษณ์ อรรถนิवास เกิดเมื่อวันที่ 18 กันยายน พ.ศ. 2519 ที่จังหวัดพิจิตร ได้รับปริญญาวิทยาศาสตรบัณฑิต สาขาจุลชีววิทยา คณะวิทยาศาสตร์ จากมหาวิทยาลัยนเรศวร จังหวัดพิษณุโลก ในปีการศึกษา 2541 และเข้ารับการศึกษาระดับปริญญาโท สาขาสัตวแพทยศาสตรบัณฑิต สาขาสัตวแพทยสาธารณสุข ภาควิชาสัตวแพทยสาธารณสุข คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ในปีการศึกษา 2550 ที่อยู่ปัจจุบัน บ้านเลขที่ 234/433 หมู่บ้านนันทวัน-ศรีนครินทร์ ซอยนันทสิริ 24 ตำบลบางเมือง อำเภอเมือง จังหวัดสมุทรปราการ 10270

