Statistical ecology

- **No variability - no statistics**
- **No uncertainty - no statistics**
- **Variability- heart of all natural phenomena**
- **Uncertainty – rule of nature**

- **Laws of uncertainty – statistical models**
- **Stage 1 – deterministic laws**
- **Stage 2- probabilistic laws**

We begin with illustration of stage1

Population Dynamics

- Single population
  - exponential growth
  - Logistic growth

- Survivorship curves

- Age/ stage structured models
  - Leslie matrix

- Two populations –Lotka Volterra models
  - Competition
  - Predation
  - Symbiosis

Understanding and Using Microbial Growth

Part I: Modeling growth –basic study

(logistic growth model and multiple regression)

Part II: Preventing growth- use of preservative

(logistic regression)

Part III: Using growth – biodegradation of pesticide

(Factorial experiment)

Part I

STATISTICAL ANALYSIS & MODELLING IN FOOD PRESERVATION

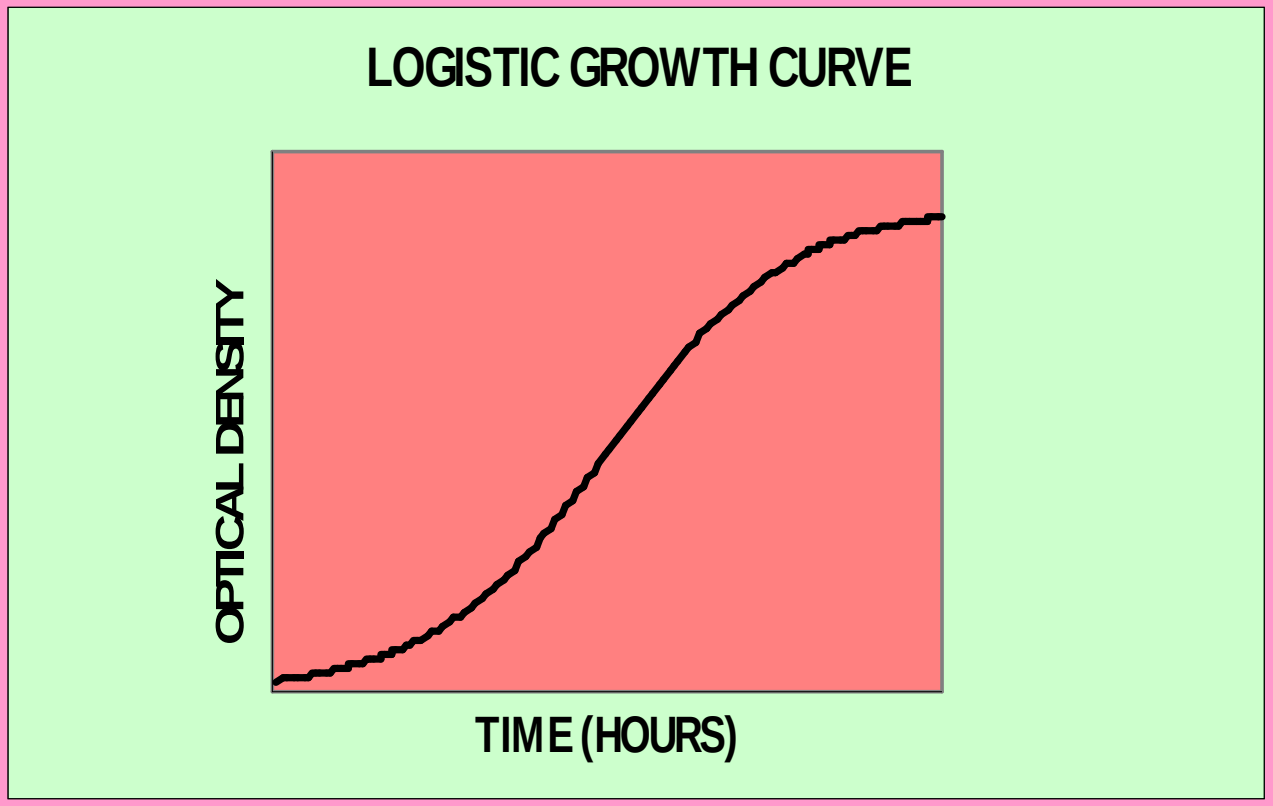
- TARGET ORGANISM: *Staphylococcus aureus* (on CORIANDER LEAVES)

- AIM: STUDY EFFECT ON GROWTH
  - pH (5LEVELS)
  - WATER ACTIVITY (Aw) (10 levels)
  - (5 x 10 = 50 COMBINATIONS)

- DATA: OPTICAL DENSITY (OD)
  - HOURLY RECORD
  - 150 HOURS
  - INCUBATED AT 37^0 C
  - Two strains: standard and wild

MODELLING STEP 1.

- **ONE pH x Aw COMBINATION**
  - **FIT LOGISTIC GROWTH CURVE**
- **$N_t = K / (1+q\, e^{-rt})$**
- **$q = (K-N_0)/ N_0$**
- **Estimate K(SATURATION LEVEL)**
- **Estimate r (GROWTH RATE)**


LOGISTIC GROWTH CURVE
OPTICAL DENSITY
TIME (HOURS)

MODELLING STEP 2.

METAMODEL

REGRESS K ON pH AND Aw

$$K = B_0 + B_1 * pH + B_2 * Aw + B_3 * Aw^2$$

REGRESS r ON pH AND Aw

VALIDATION

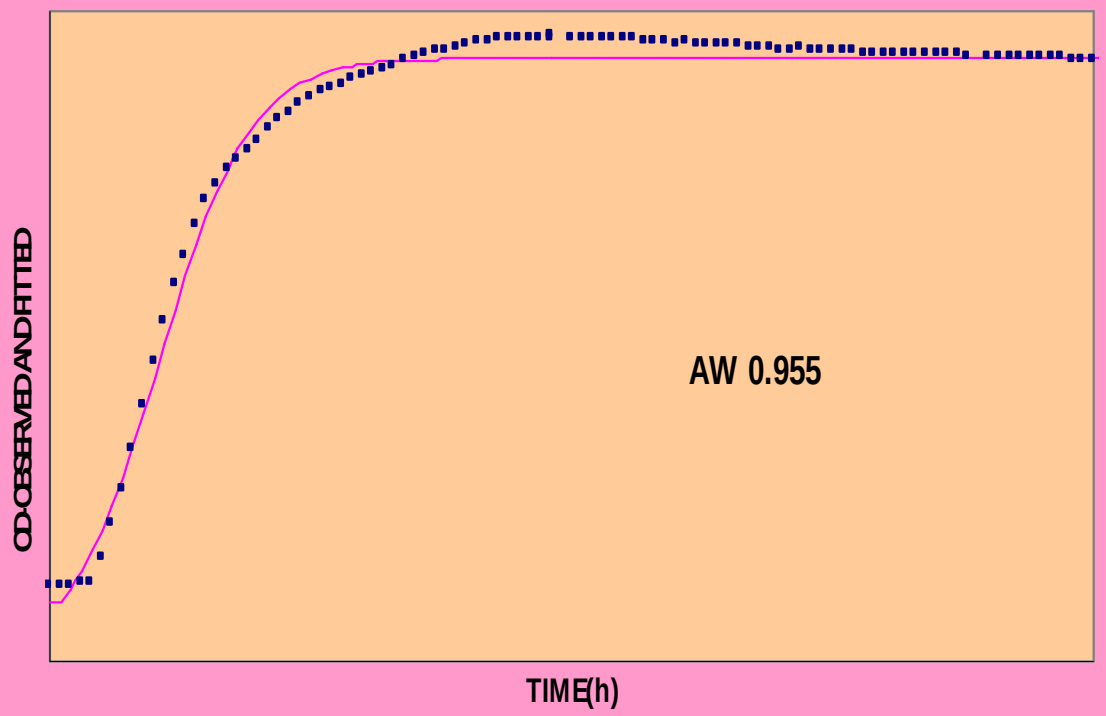
PREDICT K AND r FOR

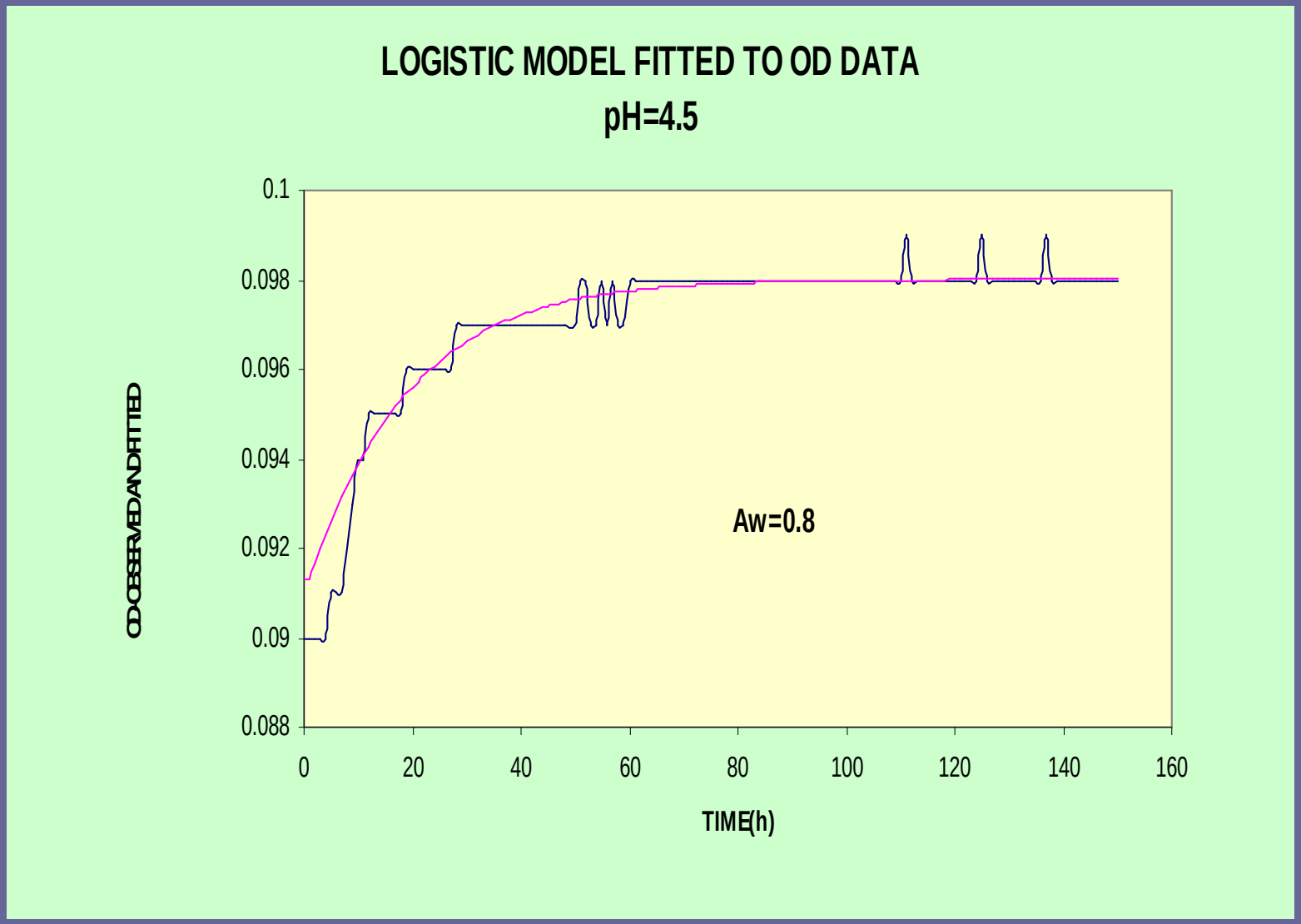
INTERMEDIATE UNUSED VALUES OF pH & Aw

CONDUCT EXPERIMENTS USING pH x Aw SPECIFIED

COMPARE OBSERVED K AND r WITH PREDICTION

RESULTS STEP 1.




LOGISTIC MODEL FITTED TO OD DATA
pH=4.5
OD OBSERVED AND FITTED
0.1
0.098
0.096
0.094
0.092
0.09
0.088
0
20
40
60
80
100
120
140
160
TIME(h)
Aw=0.8

RESULT STEP 2.

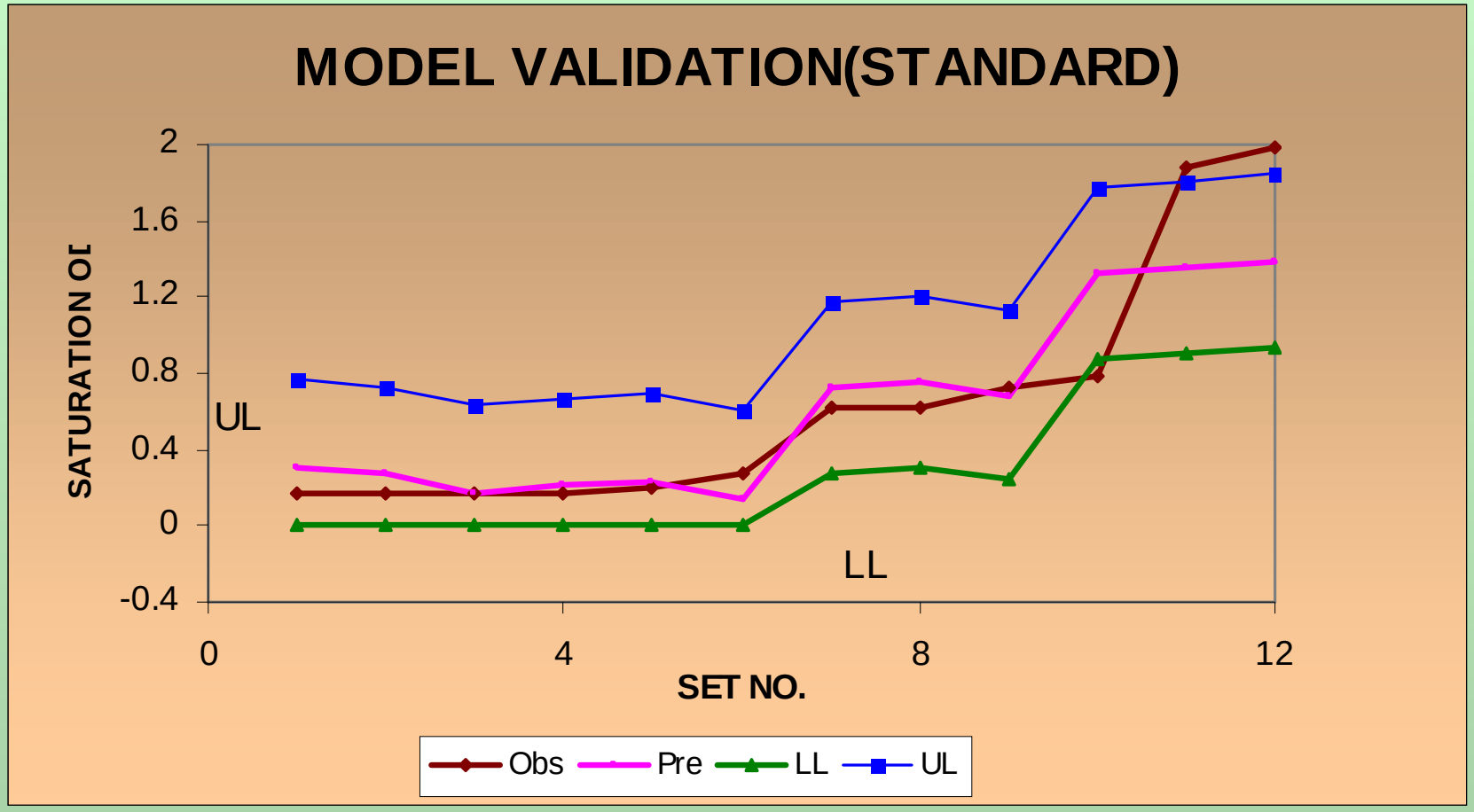
$$Kst = 55.6 + 0.0333*pH - 130*Aw + 76*Aw^2$$

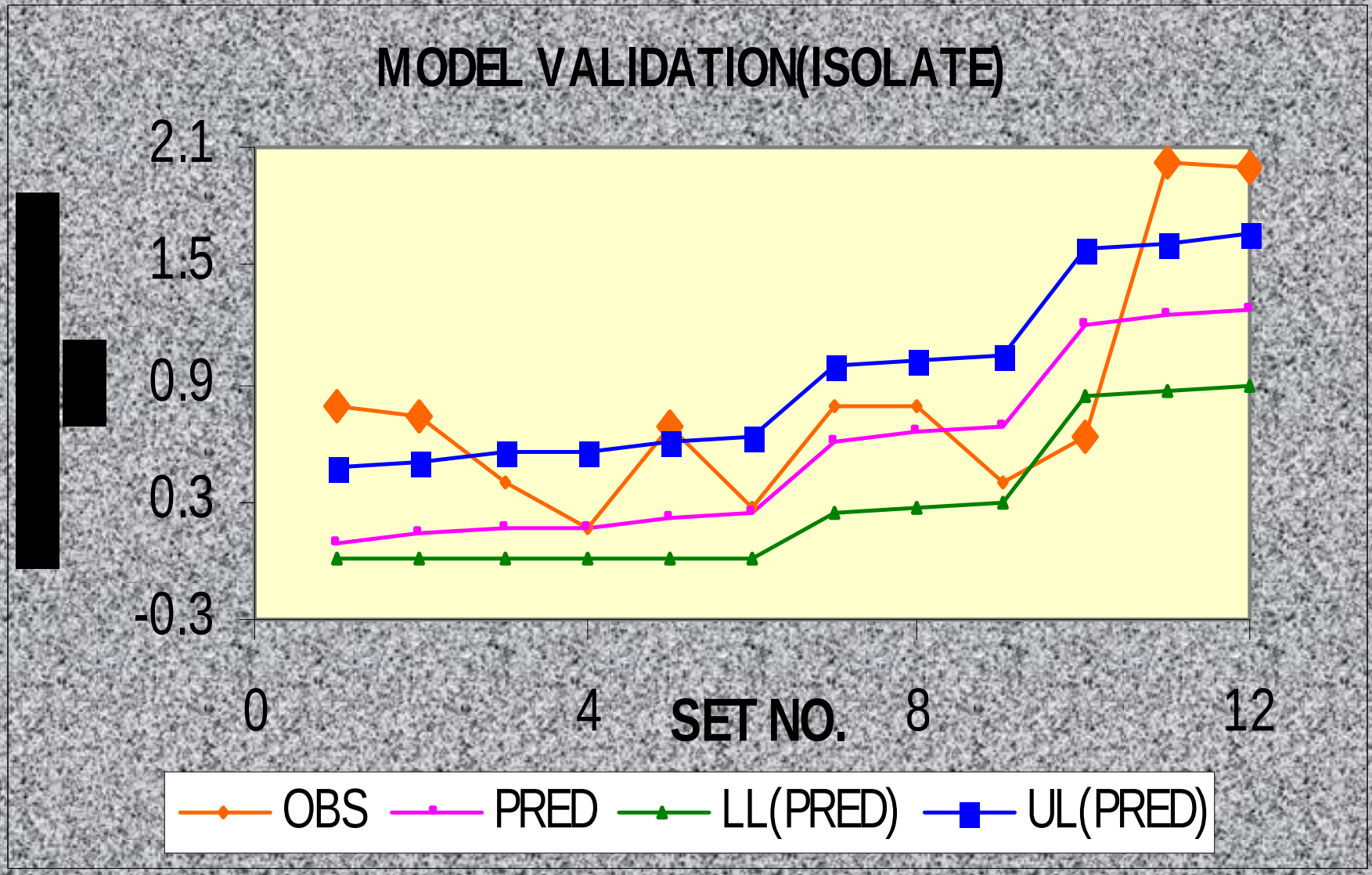
$$(R^2 = 95\%)$$

$$K_{is} = 52.1 + 0.0368*pH - 122*Aw + 71*Aw^2$$

$$(R^2 = 87\%)$$

REGRESSION FOR r – INEFFECTIVE.


MODEL VALIDATION(STANDARD)
SATURATION OI
2
1.6
1.2
0.8
0.4
0
-0.4
UL
LL
0
4
8
12
SET NO.
Obs
Pre
LL
UL


MODEL VALIDATION(ISOLATE)
2.1
1.5
0.9
0.3
-0.3
0
4
8
12
SET NO.
OBS
PRED
LL(PRED)
UL(PRED)

Conclusions

- **Logistic model fits bacterial growth data well**
- **Maximum concentration reached can be explained in terms of ambient conditions**
- **Growth rate appears to be insensitive to ambient conditions**

Part II
Preventing bacterial growth- use of preservative

Aim: Develop a ready reckoner for combination of

Aw , pH and preservative level that is safe

against a cock tail of 5 bacterial species

3 preservatives, 5 levels of each, 5 Aw levels and 6 pH levels

Effect of P.paraben on bacterial growth

			B. pumilus					*B.subtilis*			
Level	pH	Water Activity									
		1	0.98	0.96	0.93	0.9	1	0.98	0.96	0.93	0.9
0	4	3	0	1	1	0	0	0	1	0	0
	5	0	1	1	1	1	3	3	3	2	2
	6	2	3	2	2	0	3	3	3	2	1
	7	3	3	3	2	2	3	3	3	2	2
	8	0	3	3	2	2	2	3	3	0	1
	9	2	3	3	2	0	0	3	3	0	0
0.025	4	0	0	0	0	0	0	0	0	0	0
	5	0	1	0	0	0	0	0	0	0	0
	6	0	1	0	0	0	0	0	0	0	0
	7	1	1	2	2	0	0	0	0	0	0
	8	1	2	2	1	0	0	3	0	0	0
	9	2	3	3	0	0	3	3	0	0	2

Code : 0 -no growth cidal , 1- no growth cidal/static
2- no growth static, 3 - growth

Fitting Logistic regression

Results for **P. fluorescens** with preservative P. paraben

Observed	Fitted 0	1	% Correct
0	96	4	96.00%
1	8	12	60.00%
		Overall	90.00%

Variable	B	S.E.	Wald	df	Sig
LEVEL	-62.0601	17.5421	12.5159	1	.0004
PH	.7465	.2369	9.9282	1	.0016
AW	46.5984	13.4808	11.9484	1	.0005
Constant	-50.1339	13.6899	13.4110	1	.0003

Model performance

P. Paraben		P. fluorescens					
Pres	level	pH	Aw	gcode	grecode	Prob	Pred
2	0	4	1	0	0	0.36596	0
2	0	5	1	0	0	0.54907	1
2	0	6	1	3	1	0.71979	1
2	0	7	1	3	1	0.84421	1
2	0	8	1	3	1	0.91956	1
2	0	9	1	3	1	0.96018	1
2	0	4	0.98	0	0	0.1852	0
2	0	5	0.98	0	0	0.32409	0
2	0	6	0.98	3	1	0.50286	1
2	0	7	0.98	3	1	0.6809	1
2	0	8	0.98	2	0	0.81823	1
2	0	9	0.98	3	1	0.90473	1
2	0	4	0.96	0	0	0.08215	0
2	0	5	0.96	3	1	0.15882	0
2	0	6	0.96	3	1	0.28485	0
2	0	7	0.96	3	1	0.4566	0
2	0	8	0.96	3	1	0.63933	1

Using the model to build ‘safe zone’

- **Preservative: P. paraben**

- **Response**
  - **0 : if none of the 5 species grows**
  - **1 : if at least one species grows**

- **Use interpolation to generate predictions for unobserved conditions**

Ready reckoner (**Aw X Preservative level**)

pH 4
Preservative level

Aw	0	0.025	0.05	0.075	0.1	0.125	0.15	0.175	0.2	0.225	0.25	0.275	0.3
0.9	0	0	0	0	0	0	0	0	0	0	0	0	0
0.91	0	0	0	0	0	0	0	0	0	0	0	0	0
0.92	0	0	0	0	0	0	0	0	0	0	0	0	0
0.93	0	0	0	0	0	0	0	0	0	0	0	0	0
0.94	0	0	0	0	0	0	0	0	0	0	0	0	0
0.95	0	0	0	0	0	0	0	0	0	0	0	0	0
0.96	0	0	0	0	0	0	0	0	0	0	0	0	0
0.97	1	0	0	0	0	0	0	0	0	0	0	0	0
0.98	1	1	0	0	0	0	0	0	0	0	0	0	0
0.99	1	1	1	0	0	0	0	0	0	0	0	0	0
1	1	1	1	1	0	0	0	0	0	0	0	0	0

pH 7
Preservative level

Aw	0	0.025	0.05	0.075	0.1	0.125	0.15	0.175	0.2	0.225	0.25	0.275	0.3
0.9	0	0	0	0	0	0	0	0	0	0	0	0	0
0.91	0	0	0	0	0	0	0	0	0	0	0	0	0
0.92	0	0	0	0	0	0	0	0	0	0	0	0	0
0.93	0	0	0	0	0	0	0	0	0	0	0	0	0
0.94	0	0	0	0	0	0	0	0	0	0	0	0	0
0.95	1	0	0	0	0	0	0	0	0	0	0	0	0
0.96	1	1	0	0	0	0	0	0	0	0	0	0	0
0.97	1	1	1	0	0	0	0	0	0	0	0	0	0
0.98	1	1	1	1	0	0	0	0	0	0	0	0	0
0.99	1	1	1	1	1	1	0	0	0	0	0	0	0
1	1	1	1	1	1	1	1	0	0	0	0	0	0

pH 6
Preservative level

Aw	0	0.025	0.05	0.075	0.1	0.125	0.15	0.175	0.2	0.225	0.25	0.275	0.3
0.9	0	0	0	0	0	0	0	0	0	0	0	0	0
0.91	0	0	0	0	0	0	0	0	0	0	0	0	0
0.92	0	0	0	0	0	0	0	0	0	0	0	0	0
0.93	0	0	0	0	0	0	0	0	0	0	0	0	0
0.94	0	0	0	0	0	0	0	0	0	0	0	0	0
0.95	0	0	0	0	0	0	0	0	0	0	0	0	0
0.96	1	0	0	0	0	0	0	0	0	0	0	0	0
0.97	1	1	0	0	0	0	0	0	0	0	0	0	0
0.98	1	1	1	0	0	0	0	0	0	0	0	0	0
0.99	1	1	1	1	1	0	0	0	0	0	0	0	0
1	1	1	1	1	1	1	0	0	0	0	0	0	0

pH9
Preservative level

Aw	0	0.025	0.05	0.075	0.1	0.125	0.15	0.175	0.2	0.225	0.25	0.275	0.3
0.9	0	0	0	0	0	0	0	0	0	0	0	0	0
0.91	0	0	0	0	0	0	0	0	0	0	0	0	0
0.92	0	0	0	0	0	0	0	0	0	0	0	0	0
0.93	0	0	0	0	0	0	0	0	0	0	0	0	0
0.94	1	0	0	0	0	0	0	0	0	0	0	0	0
0.95	1	1	0	0	0	0	0	0	0	0	0	0	0
0.96	1	1	1	1	0	0	0	0	0	0	0	0	0
0.97	1	1	1	1	1	0	0	0	0	0	0	0	0
0.98	1	1	1	1	1	1	0	0	0	0	0	0	0
0.99	1	1	1	1	1	1	1	0	0	0	0	0	0
1	1	1	1	1	1	1	1	1	1	0	0	0	0

Safe zone contracts as Aw/pH increase

Part III

Optimizing

Biodegradation of Dimethoate

in

Industrial Effluents
by
Brevundimonas sp.

A Factorial Experiment

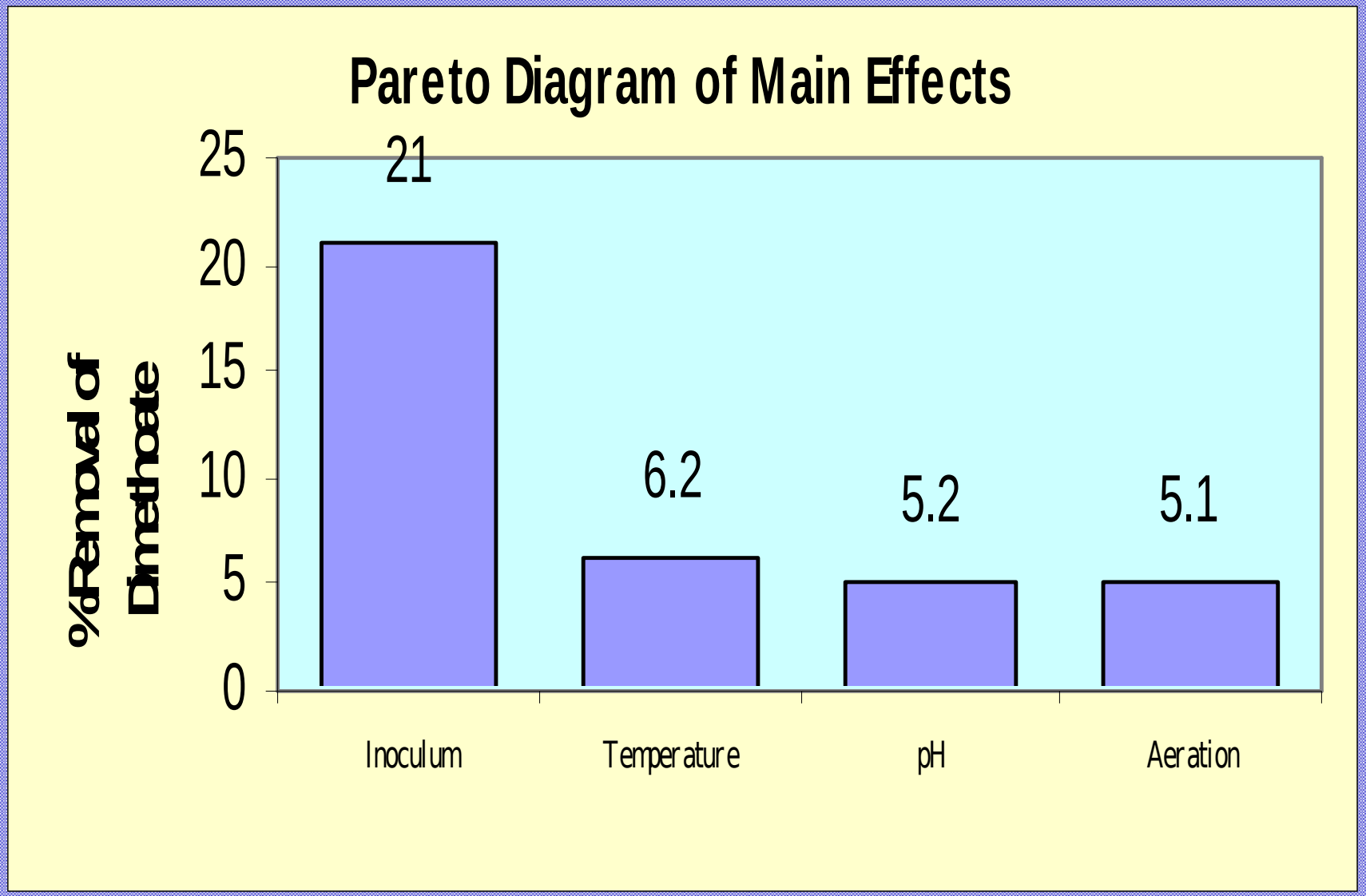
Factors and Levels

- **Temperature(T) : 30 , 40 degrees Celsius**
- **pH (p) : 5 ,7**
- **Aeration(A) : Yes , No**
- **Inoculum(I) : 10^5, 10^9 cells/ml**
- **Substrate Conc. (mg/l) (S) : 2000 , 500**
- **Total # of factor combinations $2^5=32$**
- **Response : % removal of Diamethoate**

- Initial trial 8 runs
- Quarter replicate
- Substrate effect found negligible

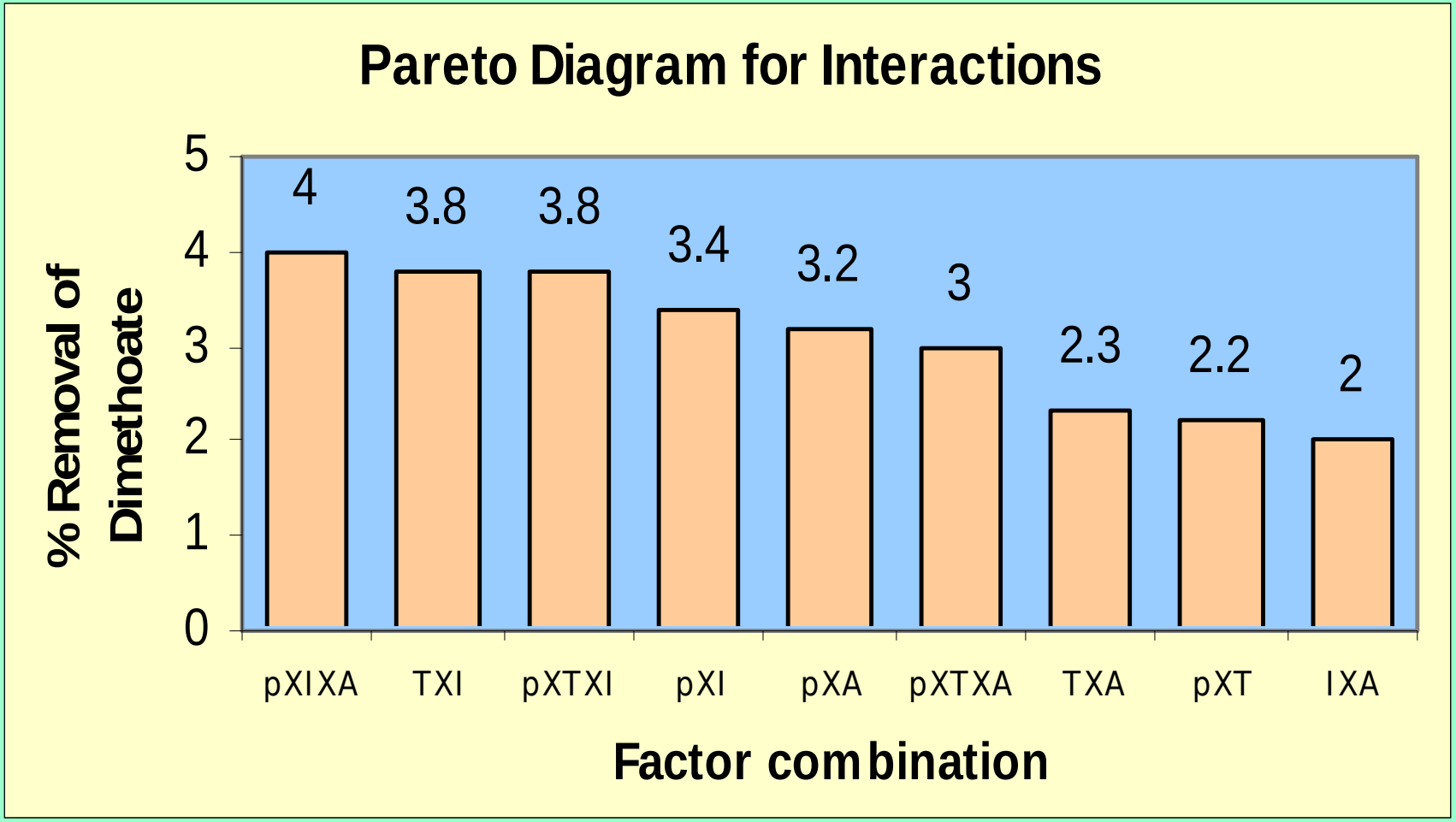
- Reduce number of factors to 4
- Combinations 16
- Second trial : Full experiment

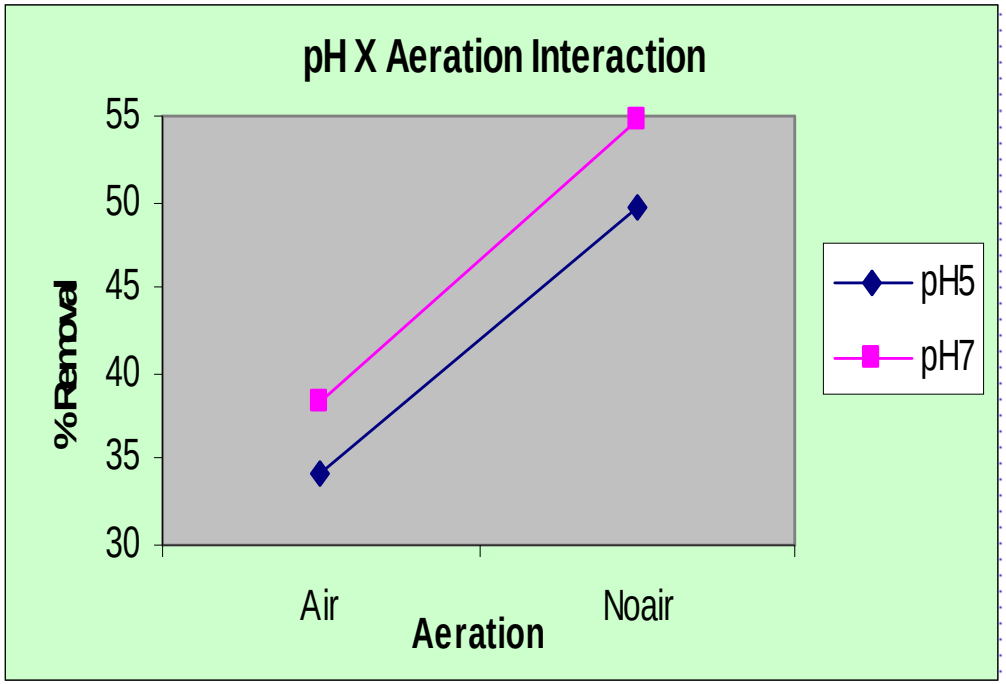
Relative Importance of Factors



Informative graph not generated by soft-wares

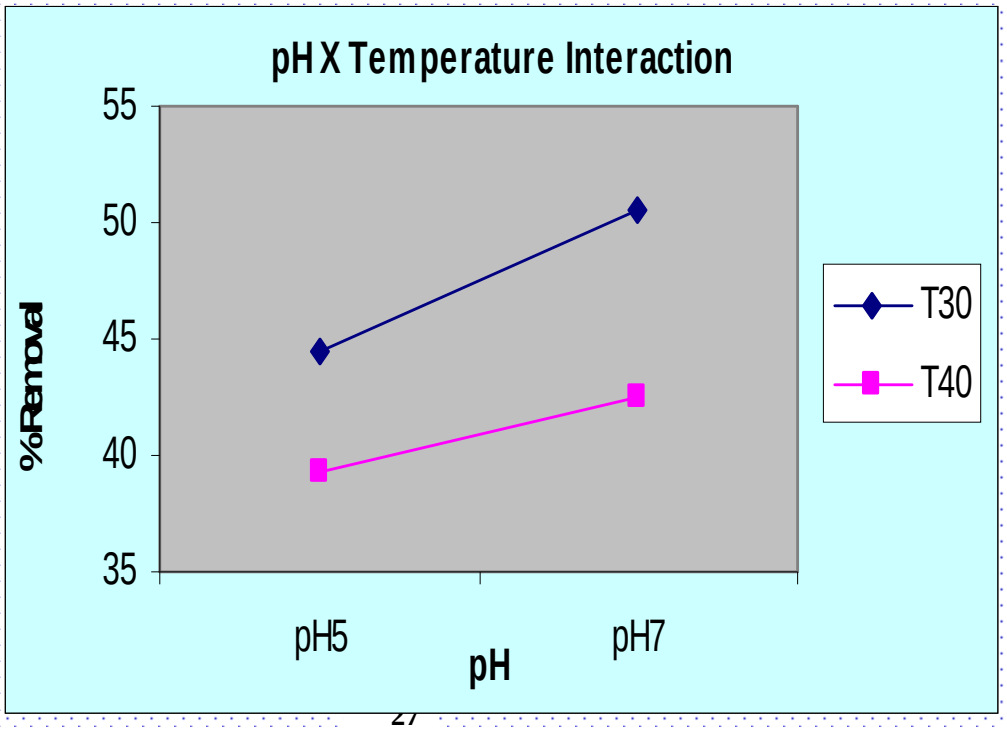
Relative Importance of Interactions





← No Interaction

Mild Interaction →



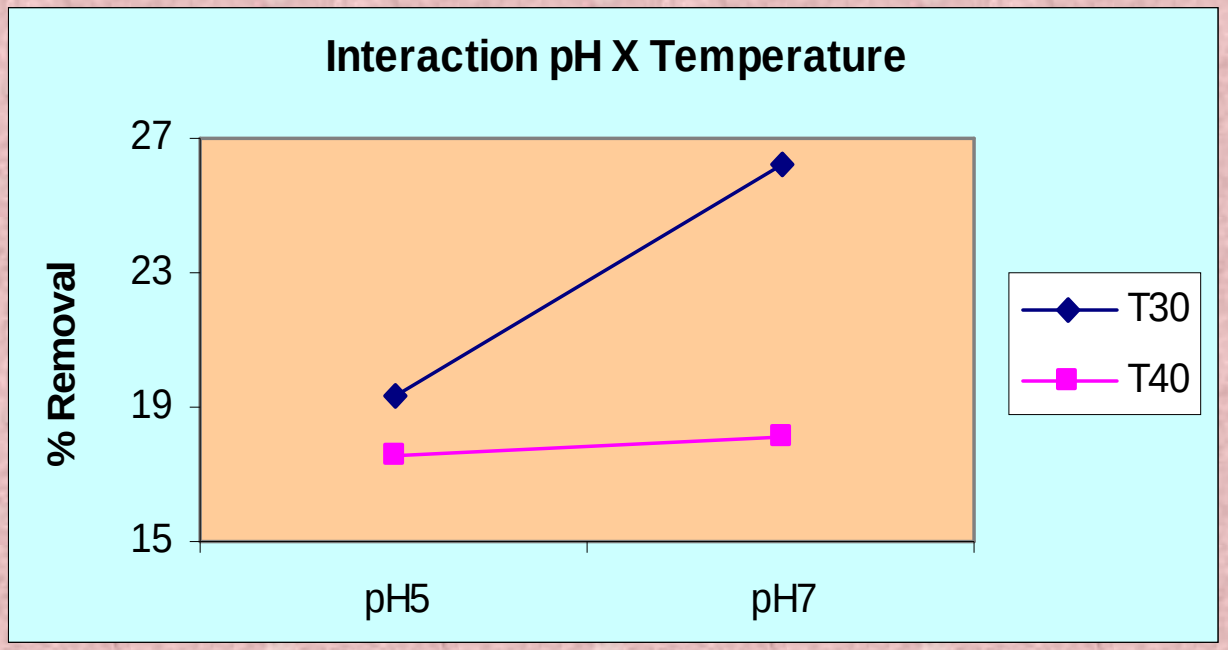
Three Factor Interaction

The Way Two Factor Interaction Depends

on

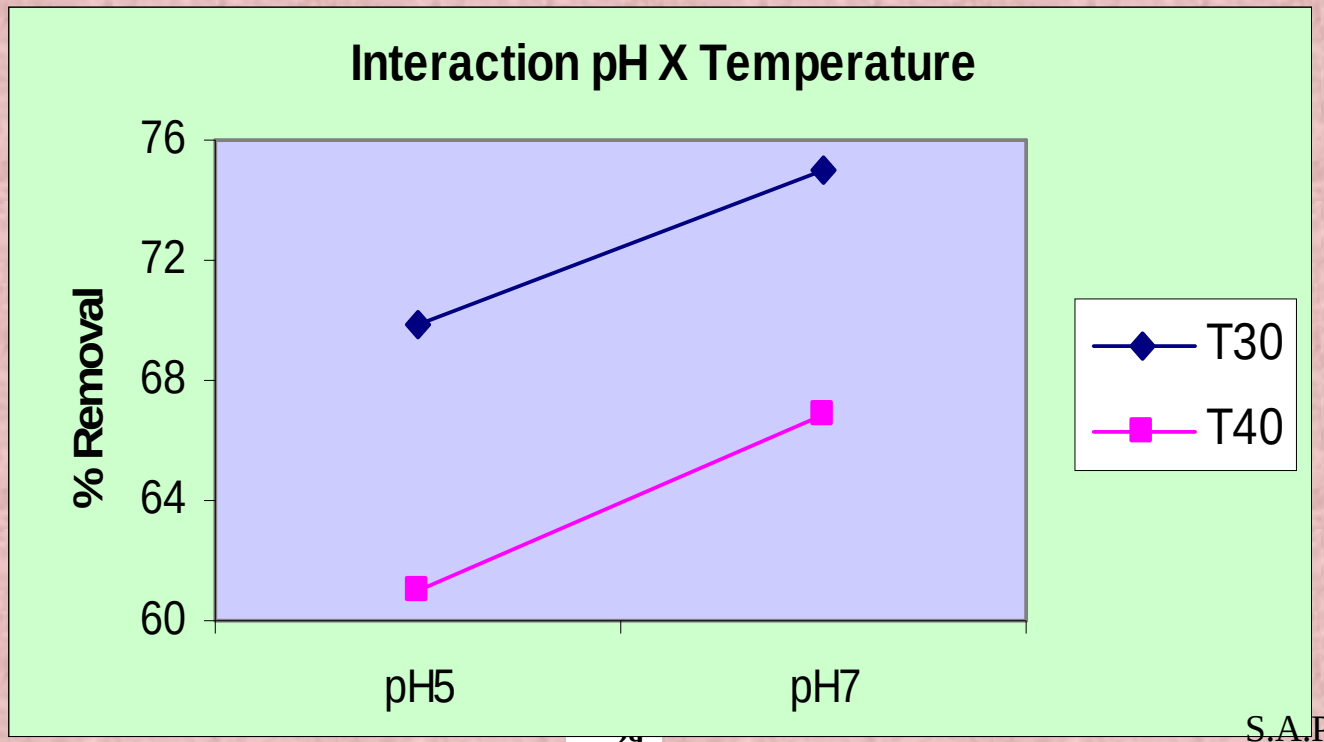
Level of Third Factor

Deserves inclusion in soft wares



Inoculum Low

Inoculum High



Choosing Best Factor Combination

pH	Temp	Inoculum	Aeration	% Removal
5	30	L	No	13.67
5	30	L	Yes	24.89
5	30	H	No	60.93
5	30	H	Yes	78.61
5	40	L	No	15.29
5	40	L	Yes	19.79
5	40	H	No	46.86
5	40	H	Yes	75.1
7	30	L	No	23.56
7	30	L	Yes	28.84
7	30	H	No	62.66
7	30	H	Yes	87.45
7	40	L	No	16.2
7	40	L	Yes	20.07
7	40	H	No	50.83
7	40	H	Yes	82.96

Follow up:

Remedial potential of a single bacterial species was found to be enhanced in a mixture