



UNIVERSITI PUTRA MALAYSIA

**EFFECTS OF pH AND ALUMINIUM ON GROWTH OF RHIZOBIA
AND THE RELATIONSHIP BETWEEN FATTY ACID COMPOSITION
OF RHIZOBIA AND ITS TOLERANCE TO LOW pH**

SHAHARAH MUHAMAD IDRIS

FSMB 1996 2

**EFFECTS OF pH AND ALUMINIUM ON GROWTH OF RHIZOBIA
AND THE RELATIONSHIP BETWEEN FATTY ACID COMPOSITION
OF RHIZOBIA AND ITS TOLERANCE TO LOW pH**

BY

SHAHARAH MUHAMAD IDRIS

**Thesis Submitted in Fulfilment of the Requirement for the Master of
Science Degree in Faculty of Food Science and Biotechnology, Universiti
Pertanian Malaysia**

1996



ACKNOWLEDGEMENTS

All praise to Allah S.W.T., who has showered me with kindness and affection during the course of my project that I cannot adequately thank for.

I would like to express my appreciation to the chairman , Assoc. Prof. Dr. Hj. Zulkifli Bin Hj. Shamsuddin and members of the supervisory committee, Dr. Arbakariya Bin Ariff and Dr. Halimi Bin Mohd. Saud for their suggestions, ideas, invaluable advice and guidance throughout this study.

Sincere thanks and gratitude are also extended to all staff of Soil Microbiology Laboratory, Department of Soil Science and staff of Fermentation Laboratory, Department of Biotechnology, for their help towards the success of this project.

I would like to express my deepest gratitude to all my family members and friends for their understanding, caring and moral support which saw me through this project.



TABLE OF CONTENTS

	PAGE
ACKNOWLEDGEMENTS	ii
LIST OF TABLES	vii
LIST OF FIGURES	ix
LIST OF PLATES.....	xii
LIST OF ABBREVIATIONS	xiii
ABSTRACT.....	xiv
ABSTRAK.....	xvii

CHAPTER

I	INTRODUCTION	1
II	LITERATURE REVIEW	6
	Rhizobia.....	6
	Functions.....	6
	Taxonomy	8
	Influence of Soil Acidity on Growth of Rhizobia	12
	Effect of pH on Mineral Nutrient Concentrations.....	12
	Effect of pH on Growth and Survival of Rhizobia ...	13
	Intracellular pH (pH _i) of Rhizobia	17
	Effect of Aluminium Toxicity	20
	Interaction between pH and Aluminium Toxicity	20
	Effect of Mineral Nutrients on Aluminium Toxicity.....	22
	Effect of Aluminium Toxicity on Growth of Rhizobia	24



	Mechanism of Aluminium Toxicity to Rhizobia.....	27
	Relationship between Fatty Acid Profiles and Acid Tolerance of Rhizobia	30
	Concluding Remarks	32
III	THEORY	35
	General Balance Equation	36
	Kinetic Models	38
	Rate of Cell Growth.....	39
	Rate of Cell Death	40
	Substrate Consumption Rate	40
	Batch Cultivation Model	41
	Model Based on Monod Equation	42
	Model Based on Gompertz Equation	46
	Continuous Cultivation Model	51
	Application of Continuous Culture for Microbial Physiology Study	53
IV	MATERIALS AND METHODS.....	55
	Microorganisms.....	55
	Culture Media for Rhizobial Strains	56
	Fermenters	57
	Chemap, CMF 100, α - Laval Fermenter.....	57
	B.Braun Baby Jar Fermenter.....	59
	Experimental Plans	60
	Cultivations	62
	Batch Culture (Shake Flask)	62
	Batch Culture (Fermenter)	63



	Continuous Culture	66
	Analytical Procedures	68
	Viable Plate Count	68
	Phosphate Determination	69
	Aluminium Determination	69
	Fatty Acid Determination	79
	Mathematical Methods: Fitting the Data.....	83
V	RESULTS AND DISCUSSIONS	85
	Cultivation in Shake Flask.....	85
	Effect of Initial Culture pH on Growth of Six Rhizobial Strains.....	85
	Growth Kinetic and Modelling.....	92
	Batch Cultivation in the Fermenter.....	99
	C _{crit} Dermination.....	99
	Testing of the Models.....	108
	Effect of Culture pH on Rhizobial Growth	108
	Effect of Aluminium Concentration on Rhizobial Growth.....	111
	Phosphate Uptake by <i>Bradyrhizobium</i> Strain TAL 102 under Different Cultivation Conditions.....	116
	Continuous Cultivation.....	122
	Time Course of Continuous Cultivation.....	122
	Effect of Aluminium on Rhizobial Growth.....	127
	Effect of Phosphate on Aluminium Toxicity.....	131
	Fatty Acid Composition in Whole Cell of Rhizobia.....	135
	Effect of Culture pHs (6.5 and 4.5) on Growth of Six Rhizobial Strains.....	135
	Fatty Acid Profile of Rhizobia.....	138
	Relationship between Fatty Acid Composition and pH Tolerance of Rhizobia.....	145
	Effect of Aluminium Concentration on Fatty Acid Composition of <i>Bradyrhizobium</i> TAL 102.....	146

VI	SUMMARY AND CONCLUSIONS.....	149
	Suggestions for Further Work.....	153
	REFERENCES.....	155
	APPENDICES.....	167
	LIST OF PUBLICATIONS.....	172
	BIOGRAPHICAL SKETCH.....	173



LIST OF TABLES

Table		Page
1	Taxonomic Classification of Rhizobia.....	9
2	Differences between Fast-Growing and Slow-Growing Rhizobia.....	10
3	Effect of pH on Growth of Several Strains of Rhizobia.....	15
4	Forms of Aluminium Potentially Present in Soil Solutions.....	21
5	Effect of Medium Composition on Aluminium Toxicity.....	23
6	Composition of Basal Medium for Cultivation of Rhizobia.....	56
7	Methods of Aluminium Determination Available in Literatures.....	71
8	Gas Chromatography Analyses of Retention Time of Standard Methyl Ester Fatty Acid.....	83
9	Comparisons of Estimates of Growth Parameters of Rhizobia Grown at Two Different Culture pHs	94
10	Comparisons of Growth Parameters Using a Modified Gompertz Equation for Acid-Tolerant <i>Bradyrhizobium</i> TAL 102 at Different Culture pHs.....	109
11	Comparisons of Growth Parameters Calculated Using a Modified Gompertz Equation for Acid-Tolerant <i>Bradyrhizobium</i> TAL 102 at Culture pH of 4.5 and Different Aluminium Concentrations.....	110
12	Comparisons of Growth Parameters for Acid-Tolerant <i>Bradyrhizobium</i> TAL 102 at Culture pH of 4.5 and Different Aluminium Concentrations in Continuous Culture.....	132



13	Maximum Cell Concentration (X_m) and (X_m/X_o) for Growth of Rhizobia at pHs 6.5 and 4.5 in Baby Jar Fermenter and Shake Flask.....	137
14	Calculated of Percentage Composition of Fatty Acid (refer to Figure 35 for the corresponding chromatograph).....	141
15	Fatty Acid Composition of <i>Rhizobium</i> and <i>Bradyrhizobium</i> spp. Cultivated at pH 6.5.....	143
16	Fatty Acid Composition of <i>Rhizobium</i> and <i>Bradyrhizobium</i> spp. Cultivated at pH 4.5.....	144
17	Fatty Acid Composition of <i>Bradyrhizobium</i> Strain TAL 102 at pH of 4.5 and Different Aluminium Concentrations in Continuous Culture (Cell harvested during steady-state was used for the analyses).....	148



LIST OF FIGURES

Figure		Page
1	General Balance Equation for Growth of Microorganisms in a Single Reactor.....	38
2	Simulation of Growth and Limiting Substrate Consumption Profile for Microorganisms According to Batch Cultivation Model Based on Monod Equation (Eq. 11 and 13).....	44
3	Effect of Substrate Concentration on Specific Growth Rate According to Monod Model (Eq. 12).....	46
4	Simulation of Growth Curves and Specific Growth Rate (μ) Profiles for Two Rhizobia, Have the Same Growth Parameters Except X_m (B) [1] and [2] Have the Same Growth Parameters Except μ_m	50
5	Schematic Diagram of 2 L Stirred Tank Fermenter (CMF 100 α -Laval).....	58
6	Flow Diagram of the Experimental Work.....	61
7	Set Up of Fermenter and Equipments for Continuous Culture Experiments.....	67
8	Standard Curves for Aluminium Determination Prepared in Distilled Water and Culture Medium.....	74
9	Effect of Changes in Medium Composition on the Concentration of Polymeric and Monomeric Aluminium Measured by Aluminon Method (Blamey et al.,	77
10	Growth of Bradyrhizobial Strain TAL 102 at pH 4.5 and 6.5.....	86
11	Growth of Bradyrhizobial Strain UPMR 29 at pH 4.5 and 6.5.....	87
12	Growth of Rhizobial Strain TAL 1826 at pH 4.5 and 6.5.....	88



13	Growth of Bradyrhizobial Str	89
14	Growth of Bradyrhizobial Strain NC 92 at pH 4.5 and 6.5.....	90
15	Growth of Rhizobial Strain TAL 1373 at pH 4.5 and 6.5.....	91
16	A Typical Decline Slope of Dissolved Oxygen Tension Against Time for C_{crit} Determination Using Dynamic Gassing-Out Technique as Proposed by Taguchi and Humprey (1986).....	101
17	Growth of <i>Bradyrhizobium</i> TAL 102 at Culture pH 3.5.....	102
18	Growth of <i>Bradyrhizobium</i> TAL 102 at Culture pH 4.0.....	103
19	Growth of <i>Bradyrhizobium</i> TAL 102 at Culture pH 4.5.....	104
20	Growth of <i>Bradyrhizobium</i> TAL 102 at Culture pH 5.5.....	105
21	Growth of <i>Bradyrhizobium</i> TAL 102 at Culture pH 6.5.....	106
22	Growth of <i>Bradyrhizobium</i> TAL 102 at Culture pH 7.5.....	107
23	Growth of <i>Bradyrhizobium</i> TAL 102 at pH 4.5 with an Average Monomeric Aluminium Activities (Σa_{Almono}) of 19.2 μ M.....	112
24	Growth of <i>Bradyrhizobium</i> TAL 102 at pH 4.5 with an Average Monomeric Aluminium Activities (Σa_{Almono}) of 45.6 μ M.....	113
25	Growth of <i>Bradyrhizobium</i> TAL 102 at pH 4.5 with an Average Monomeric Aluminium Activities (Σa_{Almono}) of 69.3 μ M.....	114
26	Profiles of Specific Rate of Phosphate Uptake (Q_p) During Growth of <i>Bradyrhizobium</i> TAL 102 at Different Culture pHs.....	120
27	Profiles of Specific Rate of Phosphate Uptake (Q_p) During Growth of <i>Bradyrhizobium</i> TAL 102 at pH 4.5 and Different Monomeric Aluminium Activities (Σa_{Almono}).....	121



28	Time Course for Growth of <i>Bradyrhizobium</i> TAL 102 in Continuous Culture at pH 4.5.....	123
29	Time Course for Growth Of <i>Bradyrhizobium</i> TAL 102 in Continuous Culture with 60 μM $\text{Al}_2(\text{SO}_4)_3$ in a Feed. Culture pH was Controlled at pH 4.5.....	124
30	Time Course for Growth of <i>Bradyrhizobium</i> TAL 102 in Continuous Culture with 320 μM $\text{Al}_2(\text{SO}_4)_3$ in a Feed. Culture pH was Controlled at pH 4.5.....	125
31	Time Course for Growth of <i>Bradyrhizobium</i> TAL 102 in Continuous Culture with 640 μM $\text{Al}_2(\text{SO}_4)_3$ in a Feed. Culture pH was Controlled at pH 4.5.....	126
32	Relationship between Steady-State Cell Concentration (X_s) and Maximum Cell Concentration (X_m) at Different Levels of Monomeric Aluminium Activities (Σa_{Almono}).....	129
33	Relationship between Phosphate Consumed by the Rhizobia and the Steady-State Cell Concentration (X_s).....	134
34	Growth of Rhizobial Strain (A) TAL 102, (C) CB 1809, Baby Jar Fermenters in Which the Culture pHs Were Controlled at pH 6.5 and 4.5 Throughout the Cultivations.....	136
35	A Typical Chromatograph Illustrating the Separation and Identification of Fatty Acid Compositions TAL 102 at pH 6.5.....	140
36	A Typical Chromatograph Illustrating the Separation and Identification of Fatty Acid Compositions TAL 102 at pH 4.5.....	140



LIST OF PLATES

Plate		Page
1	Complexation of Aluminium with Phosphate.....	117



LIST OF ABBREVIATIONS

h	:	hour
QO₂	:	Specific Oxygen Uptake (mmol O₂/g cell)
DOT	:	Dissolved Oxygen Tension
Al	:	Aluminium
P	:	Phosphate
ml	:	Millilitre
L	:	Litre

Abstract of thesis submitted to the Senate of Universiti Pertanian
Malaysia as fulfilment of the requirements for the degree of Master of Science

**EFFECTS OF pH AND ALUMINIUM ON GROWTH OF RHIZOBIA
AND THE RELATIONSHIP BETWEEN FATTY ACID COMPOSITION
OF RHIZOBIA AND ITS TOLERANCE TO LOW pH**

BY

SHAHARAH MUHAMAD IDRIS

JULY 1996

Chairman : Associate Professor Zulkifli Hj. Shamsuddin,

Faculty : Food Science and Biotechnology

The effect of two different initial culture pHs on growth of six strains of rhizobia (TAL 102, was first studied by using shake flask experiment. Only *Bradyrhizobium* TAL 102 grew better at pH 4.5 compared to 6.5 and this strain was chosen as an acid-tolerant rhizobia. This result is in agreement with the result of the experiment using the fermenter in which the culture pH was controlled at a constant value of 4.5 and 6.5 throughout the cultivation. However,



parameters such as maximum cell concentration attained (X_m) differed significantly.

A study on the effects of different culture pHs and aluminium (Al) concentrations on growth of acid-tolerant rhizobia (*Bradyrhizobium* TAL 102) was carried out using a 2 L stirred tank fermenter. A modified Gompertz equation was found to be sufficient in modelling growth of rhizobia at two different initial culture pHs (shake flask experiment) and also growth of TAL 102 at different pH levels and Al concentrations. The growth parameters (X_{max} , μ_{max} and λ) of acid-tolerant rhizobia under different culture pHs and Al concentrations were calculated using the model. Maximum cell concentration (X_{max}) value was highest at pH 4.5 with a drastic reduction at pH below 4. Although the maximum specific growth rate (μ_m) was reduced at pH 4 and below, the effect was not clear for growth at pH 4.5 and above. The presence of monomeric Al activity (Σa_{Almono}) reduced X_{max} significantly but the λ and μ_{max} were not significantly affected. The X_{max} , μ_{max} and λ for growth of TAL 102 at pH 4.5 with $45.6 \mu M \Sigma a_{Almono}$ were 2.0×10^9 cfu/ml, 0.015 h^{-1} and 5 h, respectively.

The effect of Al concentration on growth of rhizobia (TAL 102) was also investigated using continuous (chemostat) culture. The relationship between Σa_{Almono} and steady-state cell concentration (X_s) can be presented in the form of



$\ln [\Sigma a_{Almono}] = 6.53 - 0.101[X_s]$, indicating a decrease in rhizobial cell concentration with increased in toxicity of Σa_{Almono} .

The composition of fatty acids of rhizobia was successfully separated and analysed using Gas Chromatography technique. The major proportions of fatty acids present in all rhizobia studied were C:16 to C:20. From this study it was observed that the tolerance of rhizobia to low pH can be related to high proportions of C:18 and C:20. The C:18 fatty acids of *Bradyrhizobium* TAL 102, which was the major proportion of fatty acid when grown at pH 4.5 in the presence of Al, increased significantly with increasing concentration of Al in the culture. Although the proportions of other fatty acids such as C:12 and C:20 changed with increasing Al level, a significant relationship to pH tolerance of rhizobia was not evident. It was suggested that C:18 fatty acids played a role in rhizobial tolerance to Al.

Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian
Malaysia sebagai memenuhi keperluan Ijazah Master Sains.

**PENGARUH pH DAN ALUMINIUM TERHADAP RHIZOBIA DAN
PERKAITAN DIANTARA KOMPOSISI ASID LEMAK RHIZOBIA
DENGAN TOLERANSI KEPADA pH RENDAH**

OLEH

SHAHARAH MUHAMAD IDRIS

JULAI 1996

Pengerusi : Profesor Madya Zulkifli Hj. Shamsuddin, Ph.D.

Fakulti : Sains Makanan dan Bioteknologi

Kesan dua kultura pH pemula ke atas pertumbuhan enam strain rhizobia (TAL 102, UPMR 29, CB 1809, NC 92, TAL 1826 dan TAL 1373) telah dikaji dengan menggunakan kelalang goncang. Hanya *Bradyrhizobium* TAL 102 menunjukkan pertumbuhan yang baik pada pH 4.5 berbanding pH 6.5 dan strain ini telah dipilih sebagai rhizobia toleran asid. Keputusan ini adalah bertepatan dengan keputusan yang diperolehi daripada eksperimen menggunakan fermenter di mana semasa pertumbuhan, pH dikawal pada pH 4.5 dan 6.5.



Walaupun bagaimanapun, parameter pertumbuhan contohnya kepekatan sel maksimum tercapai (X_m) didapati mempunyai perbezaan yang ketara.

Kajian ke atas kesan pH kultura dan kepekatan aluminium (Al) terhadap pertumbuhan rhizobia toleran asid (*Bradyrhizobium* TAL 102) telah dijalankan menggunakan fermenter berpengaduk 2 L yang dilengkapi dengan sistem kawalan pH. Persamaan Gompertz terubahsuai didapati sesuai dalam memodelkan pertumbuhan rhizobia dalam keadaan pH pemula kultura yang berbeza (eksperimen kelalang goncang) dan juga pertumbuhan TAL 102 pada tahap pH dan kepekatan Al yang berbeza. Parameter pertumbuhan (X_{max} , μ_{max} dan λ) oleh rhizobia toleran asid dalam kultura pH dan kepekatan Al yang berbeza, telah dikira menggunakan model tersebut. Nilai kepekatan sel yang maksima (X_{max}) adalah tertinggi pada pH 4.5 dan menurun secara mendadak di bawah pH 4. Walaupun kadar pertumbuhan maksima (μ_m) adalah rendah pada pH 4 dan ke bawah, kesannya adalah tidak jelas pada pH 4.5 dan ke atas. Kehadiran aktiviti Al monomerik (Σa_{Almono}) telah menurunkan X_{max} , dengan ketara, tetapi tidak memberi kesan yang bererti kepada λ dan μ_{max} . Nilai X_{max} , μ_{max} dan λ untuk pertumbuhan TAL 102 pada pH 4.5 dengan Σa_{Almono} pada 45.6 μM adalah 2.0×10^9 cfu/ml, 0.015 j^{-1} dan 5 j berturutan.



Kesan kepekatan Al ke atas pertumbuhan rhizobia (TAL 102) juga telah dikaji menggunakan kultura selanjar. Perhubungan antara Σa_{Almono} dan fasa pegun bilangan sel (X_s) boleh ditunjukkan dalam bentuk $\ln [\Sigma a_{Almono}] = 6.53 - 0.101 [X_s]$, di mana menunjukkan penurunan bilangan sel dengan meningkatnya ketoksikan Σa_{Almono} .

Komposisi asid lemak rhizobia telah dianalisis dengan menggunakan teknik Kromatografi Gas. Asid lemak utama yang hadir dalam semua rhizobia yang telah dikaji didapati dari C:16 hingga C:20. Daripada kajian ini didapati toleransi rhizobia pada pH yang rendah boleh dikaitkan dengan peningkatan kandungan asid lemak C:18 dan C:20. Asid lemak C:18 *Bradyrhizobium* TAL 102, yang paling tinggi apabila dikulturkan pada pH 4.5 dengan kehadiran Al, mengalami peningkatan dengan meningkatnya kepekatan Al di dalam kultur. Walaupun kandungan rantaian karbon lain contohnya C:12 dan C:20 mengalami perubahan dengan pertambahan Al, perkaitan bererti dengan toleransi rhizobia kepada pH adalah tidak nyata. Adalah dicadangkan bahawa rantaian asid lemak C:18 memainkan peranan bererti dalam toleransi rhizobia kepada Al.



CHAPTER I

INTRODUCTION

Soil acidity is a major factor which limits legume growth and nitrogen fixation because of its adverse effects on growth of the host plant, root nodule bacteria namely *Rhizobium* and *Bradyrhizobium* and the symbiotic process (O'Hara et al., 1989). Successful symbiotic associations between legumes and their root nodule bacteria are of immense importance both in agriculture and forest ecosystem. *Rhizobium*-legume symbiosis is a highly specific interaction (Glenn and Dilworth, 1991) and is influenced by the environment, such as soil pH and Al concentration. Soil acidity is a complex of high proton concentration and its interaction with various mineral ions. It was found that as the pH decreases below 5 the concentration of soluble Al increases. The degree of Al tolerance in rhizobia appears to be a stable genetic character, implying some underlying physiological or biochemical differences between Al-tolerant and Al-sensitive strains which may be attributed to the synthesis of specific enzymes.

Growth of rhizobia is influenced by the physical properties and chemical composition of soils (Ayanaba et al., 1983; Keyser and Munns, 1979(a,b)).



Acidity and Al toxicity are the major soil factors which limit growth of rhizobia. The influence of pH (Date and Halliday, 1978; Shamsuddin, 1987), Al (Ayanaba et al., 1983; Keyser and Munns, 1979 (a,b); Shamsuddin, 1987), phosphate (Ayanaba et al., 1983; Cassman et al., 1981) and calcium (O'Hara et al., 1989) concentrations on growth of rhizobia have been extensively studied. The relationship between Al toxicity and culture pH; and also its interaction with other minerals in soils have also been investigated (Ayanaba et al., 1983; Cassman et al., 1981; Date and Halliday, 1978; Keyser and Munns, 1979(a,b); O'Hara et al., 1989). However, interpretation of data available from these types of experiments is difficult because it involved many variables and parameters. In addition, many sets of experiment have to be carried out in order to get more realistic result on the effect of each chemical component and other growth variables. Furthermore, if the result is not presented in a concise form or in the form of quantifiable values, comparison of data with others available in the literature is difficult.

Most studies on the effect of pH and Al on growth of bradyrhizobia have been carried out in batch culture using shake flask without automatic pH control system. In many cases, the culture pH was presumably controlled using biological buffers. Once growth proceeded the buffers may be metabolised and compound such as ammonia will be liberated which leads to a reduction in the buffering capacity and a significant increase in culture pH. Interpretation of data available

from this type of experiment is difficult because comparison was normally made between cultures cultivated at different pH levels with or without Al, whereby the culture pH and hence, monomeric Al has changed during growth. Small changes in culture pH alone could significantly affect the growth of rhizobial cultures (Richardson and Simpson, 1989; Thornton and Davey, 1983 (a,b)). In order to get more realistic result, the experiment should be conducted using the fermenter with an automatic pH control system.

Microorganisms can adapt to different changes in the environment by modifying their membranes (Heipieper et al., 1994). Changes in the fatty acid composition of membrane lipids are the most common reaction of bacteria against membrane active substances. Currently there is no documented evidence on the identification of specific fatty acid induced or repressed by acidity. Several possible starting points are evident in the literature including the production of shorter carbon chain fatty acid in acid-tolerant strains of rhizobia (Shaharah, 1993). The significance of fatty acid composition in characterising acid-tolerant and intolerant strains is still unknown. If the relationship between pH tolerance and fatty acid composition is known, this will assist in developing simple techniques for screening of acid-tolerant rhizobial strain.

The objectives of this research study, based on the problems related to the relationships between pH and Al concentration on growth and survival of rhizobia and the possible methods overcoming these problems, are as follows:

- 1) To investigate the effect of pH on growth and survival of several rhizobial strains in shake flask culture without the addition of buffer to control culture pH at the required level.
- 2) To investigate the effect of pH and Al on growth of acid-tolerant rhizobia (selection based on results from shake flask experiment) in batch cultivation using fermenters with automatic pH controls.
- 3) To develop a simple mathematical model for describing growth of rhizobia in biological terms under different cultivation conditions.
- 4) To investigate the effect of monomeric Al activity (Σa_{Almono}) on growth and survival of rhizobia in continuous culture with pH control in which steady state of nutrients in the culture medium can be achieved.
- 5) To determine the relationship between fatty acid compositions of rhizobia and their tolerance to low pH.

In the present study, a quick selection of acid-tolerant bradyrhizobia was carried out using shake flask cultures. The selected strain was then used for the subsequent experiment to study growth of acid-tolerant rhizobia at different pH levels and Al concentrations using a fermenter with automatic pH control system.

The experimental data obtained was used to explain the mechanism of the interaction between pH and Al on growth of rhizobia. The identified acid-tolerant strain was then used for subsequent continuous cultivation experiments in which the culture pH and Al concentration were controlled at required values while other cultivation conditions such as organic materials and phosphate levels were kept constant. This highly controlled cultivation conditions enabled investigations on the effect of Al toxicity on growth of *Bradyrhizobium* under different conditions which could be used to extrapolate results from laboratory to soil conditions. The Gompertz equation was used to verify the experimental data under different cultural conditions in which various parameter values such as maximum cell population, maximum specific growth rate and lag phase were calculated. The modelling was aimed at simulating the growth pattern so that a prediction of growth under different cultural conditions could be logically constructed. Although work on the influence of pH and Al on growth of rhizobia have been studied extensively, no models has been proposed to describe the result in a concise form.

The composition of free fatty acid in all rhizobia employed in this study was analysed using gas chromatography. The changes in fatty acid composition under different cultivation conditions (different pHs and Al concentrations) was also investigated.