

UTILIZATION OF WHEY FOR THE PRODUCTION OF
SOME BIOLOGICAL SUBSTANCES

BY
OSMAN ABDEL-ALEEM ABDEL-LATEEF



A thesis submitted in partial fulfillment
of
the requirements for the degree of



637.147
C. A

MASTER OF SCIENCE

in
Agriculture
(Dairy Science and Technology)

37328

Department of Food Science
Faculty of Agriculture
Ain Shams University

1991

Approval Sheet

UTILIZATION OF WHEY FOR THE PRODUCTION OF
SOME BIOLOGICAL SUBSTANCES

BY

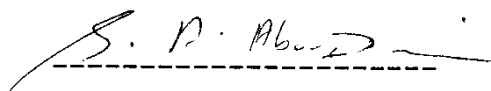
OSMAN ABDEL-ALEEM ABDEL-LATEEF

B.Sc.Agric.(Dairy Sci. and Tech.) Ain Shams Univ., 1984

This thesis for M.Sc. degree has been approved by:

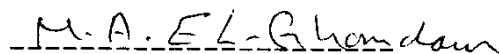
Prof. Dr. S. A. ABOU-DONIA

Prof. of Dairy Sci. and Tech.
Fac. of Agric., Alex. Univ.



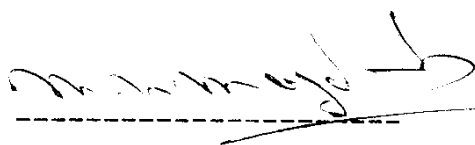
Prof. Dr. M.A.A. EL GHANDOUR

Prof. of Dairy Sci. and Tech.
Fac. of Agric., Ain Shams Univ.



Prof. Dr. M.N.I. MAGDOUB

Prof. of Dairy Sci. and Tech.
Fac. of Agric., Ain Shams Univ.



Date of examination: 15 / 7 / 1991.



UTILIZATION OF WHEY FOR THE PRODUCTION OF
SOME BIOLOGICAL SUBSTANCES

BY

OSMAN ABDEL-ALEEM ABDEL-LATEEF

B.Sc.Agric. (Dairy Sci. and Tech.), 1984
Ain Shams University

Under the Supervision of:

- Prof. Dr. M. N. I. MAGDOUB
Prof. of Dairy Science and Technology
Fac. of Agric., Ain Shams University.
- Prof. Dr. NAGWA E. SULTAN
Prof. of Dairy Science and Technology
Fac. of Agric., Ain Shams University.

ABSTRACT

This study was implemented to investigate characteristics (growth, O.D., growth rate (u) and doubling time, t.d) and productivity (biomass amount g/l, vitamin B12 yield mg/l, B12/biomass ratio, utilized lactose % and the culture final BOD) of five strains belonging to Propionibacterium freudenreichii Subsp.freudenreichii (EMC 1137), P.freudenreichii Subsp.Shermanii (EMC 1140, 1163), and P.jensenii (EMC 1142, 1143) grown in whey permeate containing 5 mg/l cobalt chloride at 30°C for 11 day (264 h). Strains, P.freudenreichii Subsp.Shermanii EMC 1140 and EMC 1163 proved to have the highest growth activity and productivity.

Besides, effect of some nutritional additives on activity and productivity of both strains (P.freudenreichii Subsp.Shermanii EMC 1140 and EMC 1163), which were grown in whey permeate containing 5 mg/l cobalt chloride and 15 ppm 5, 6 dimethylbenzimidazole, were studied.

The results obtained indicate that the preferable concentrations of these nutritional additives to be added to whey permeate, were 1 , 1.5% yeast extract; 15.0, 15.0% kefir whey, 0.25, 0.25% choline chloride, 0.25, 0.25% glutamic acid, 0.25, 0.25% rape seed extract and 0.25, 0.75% sodium lactate, for both strains, respectively. Such results could be considered as a good guide to utilize cheese whey as a bacterial medium for biomass production and B12 biosynthesis.

ACKNOWLEDGEMENT

The author would like to express his deepest gratitude and sincere appreciation to Prof. Dr. M. N. I. MAGDOUB and Prof. Dr. NAGWA E. SULTAN, Professors of Dairy Science; and Dr. E.O. FAYED, Associate Professor of Dairy Science, Food Science Department, Faculty of Agriculture, Ain Shams University; for their guidance, valuable suggestions, encouragement and very possible help, they kindly offered throughout the course of this work.

Deep gratitude would be expressed to Cairo Microbiological Resource Center (MIRCEN) and Ultrafiltration Unit, Food Science Department, Faculty of Agriculture, Ain Shams University; and Prof. Dr. AZZA ESMAEL, Prof. of Dairy Science, National Research Centre.

Many thanks are also due to all staff members of Food Science Department, Ain Shams University, for their great help and encouragement.

	<u>Page</u>
<u>C O N T E N T S</u>	
- INTRODUCTION	1
- REVIEW OF LITERATURE	3
I. Importance of Vitamin B ₁₂	3
II. Microbial Vitamin B ₁₂	4
A. Single strain cultures	4
B. Mixed strain cultures	10
III. Microbial productivity of biomass and Vitamin B ₁₂	15
A. Nutritional requirements	15
1- Carbon source	16
2- Minerals	19
3- Amino acids and vitamins	21
4- Vitamin B ₁₂ precursors	23
B. Physical factors	28
1- Aeration	28
2- Temperature	30
3- Acidity (pH)	31
4- Light	32
- MATERIALS AND METHODS	34
I. Materials	34
A. Microbial strains	34
B. Standard Inoculum	34
C. Standard Fermentation Medium (SFM)	35
D. Additives	35

	<u>Page</u>
II. Experimental Procedures	36
A. Strain selection	36
B. Nutritional additives	37
III. Methods	38
A. Growth curve	38
B. Biomass amount	39
C. Vitamin B ₁₂ assay	39
D. Protein content	40
E. Lactose content	40
F. Biochemical oxygen Demand (BOD)	41
G. Statistical analysis	42
- RESULTS AND DISCUSSION	43
PART I: STRAIN TRAITS	43
A. Growth characteristics	43
B. Strains Productivity	51
PART II: EFFECT OF SOME NUTRITIONAL ADDITIVES	56
A. Effect of choline chloride	56
B. Effect of glutamic acid	63
C. Effect of rape seed extract	69
D. Effect of sodium lactate	75
E. Effect of yeast extract	81
F. Effect of kefir whey	87
- SUMMARY AND CONSLUSION	93
- REFERENCES	98
- ARABIC SUMMARY	--

LIST OF TABLES

Page

No.

- 1 Log of growth intensity as optical density(O.D) of five strains belonging to Propionibacterium freudenreichii (EMC 1137, 1140, 1163) and P.jensenii (EMC 1142, 1143), grown in whey permeate at 30 C for 11 day (264 h). 44
- 2 Growth rate (u) and doubling time (t.d)of five strains belonging to Propionibacterium freudenreichii (EMC 1137, 1140, 1163) and P.jensenii (EMC 1142, 1143) grown in whey permeate at 30 C for 11 day (264 h). 47
- 3 TSRT results obtained for average growth rate (u) and doubling time (t.d) of five strains belonging to Propionibacterium freudenreichii (EMC 1137, 1140, 1163) and P.jensenii (EMC 1142,1143) grown in whey permeate at 30 C for 11 day (264 h). 49
- 4 Biomass B₁₂ production, B₁₂/biomass ratio, utilized lactose and final BOD by five strains belonging to Propionibacterium freudenreichii (EMC 1137, 1140, 1163) and P.jensenii (EMC 1142, 1143) grown in whey permeate at 30 C for 11 day (264h) 52
- 5 TSRT results obtained for different criteria of five strains belonging to Propionibacterium freudenreichii EMC 1137, 1140, 1163) and P.jensenii (EMC 1142, 1143) grown in whey permeate at 30 C for 11 day (264 h). 54
- 6 Biomass amount, B₁₂ yield, B₁₂/biomass ratio, utilized lactose and final BOD of Propioni-

No.		Page
	<u>bacterium freudenreichii</u> Subsp. <u>Shermanii</u> (EMC 1140 and EMC 1163)grown in whey permeate containing different concentrations of choline chloride at 30 C for 11 day (264 h).	57
7	Probability (Pr F) parameters of GLM statistical analysis for different criteria of <u>Propionibacterium frendenreichii</u> subsp. <u>shermanii</u> (EMC 1140 and EMC 1163) grown in whey permeat containing different concentrations of choline chloride at 30 C for 11 day (264 h).	59
8	TSRT results obtained for effects of choline chloride concentrations on different criteria of <u>Propionibacterium freudenreichii</u> Subsp. <u>Shermanii</u> EMC 1140 and EMC 1163, grown in whey permeate at 30 C for 11 day (264 h).	60
9	Biomass amount, B ₁₂ yield, B ₁₂ /biomass ratio, utilized lactose and final BOD of <u>probionibacterium freudenreichii</u> Subsp. <u>Shermanii</u> EMC 1140 and EMC 1163 grown in whey permeate containing different concentrations of glutamic acid, at 30 C for 11 day (264 h).	64
10	Probability (Pr F) parameters of GLM statistical analysis for different criteria of <u>Propionibacterium freudenreichii</u> Subsp. <u>Shermanii</u> grown in whey permeate containing different concentrations of glutamic acid at 30 C for 11 day (264 h).	66

	<u>Page</u>
No.	
11	TSRT results obtained for effects of glutamic acid concentrations on different criteria of <u>Propionibacterium freudenreichii</u> Subsp. <u>Shermanii</u> EMC 1140 and EMC 1163, grown at 30 C for 11 day in whey permeate. 67
12	Biomass amount, B ₁₂ yield, B ₁₂ /biomass ratio, utilized lactose, and the final BOD of <u>Propionibacterium freudenreichii</u> Subsp. <u>Shermanii</u> grown in whey permeate containing different concentrations of rape seed extract at 30 C for 11 days (264 hr). 70
13	Propability (Pr F) parameters of GLM statistical analysis for different criteria of <u>Propionibacterium freudenreichii</u> Subsp. <u>Shermanii</u> grown in whey permeate containing different rape seed extract of concentrations at 30 C for 11 day (264 h). 72
14	TSRT results obtained for effects of rape seed extract concentrations on different average criteria of <u>Propionibacterium freudenreichii</u> Subsp. <u>Shermanii</u> EMC 1140 and EMC 1163, grown at 30 C for 11 day in whey permeate. 73
15	Biomass amount, B ₁₂ yield, B ₁₂ /biomass ratio, utilized lactose and the final BOD of <u>Propionibacterium freudenreichii</u> Subsp. <u>Shermanii</u> EMC 1140 and EMC 1163 grown in whey permeate containing different concentrations of sodium lactate at 30 C for 11 day (264 h). 76

No.		Page
16	Probability (Pr F) parameters of GLM statistical analysis for different criteria of <u>Propionibacterium freudenreichii</u> Subsp. <u>Shermanii</u> grown in whey permeate containing different concentrations of sodium lactate at 30 C for 11 day (264 h).	78
17	TSRT results obtained for the different effects of sodium lactate concentration on different average criteria of <u>Propionibacterium freudenreichii</u> Subsp. <u>Shermanii</u> EMC 1140 and EMC 1163, grown at 30 C for 11 days in whey permeate.	79
18	Biomass amount, B ₁₂ yield, B ₁₂ /biomass ratio, utilized lactose and the final BOD of <u>Propionibacterium freudenreichii</u> Subsp. <u>Shermanii</u> EMC 1140 and EMC 1163 grown in whey permeate containing different concentrations of yeast extract at 30 C for 11 day (264 h).	82
19	Probability (Pr F) parameters of GLM statistical analysis for different criteria of <u>Propionibacterium freudenreichii</u> Subsp. <u>Shermanii</u> growing in whey permeate containing different concentrations of yeast extract at 30 C for 11 day (264 h).	84
20	TSRT results obtained for the different effects of yeast extract concentration on different average criteria of <u>Propionobacterium freudenreichii</u> Subsp. <u>Shermanii</u> EMC 1140 and EMC 1163, grown at 30 C for 11 days in whey permeate.	85

No.		Page
21	Biomass amount, B_{12} yield, B_{12} /biomass ratio, utilized lactose and the final BOD of <u>Propionibacterium freudenreichii</u> Subsp. <u>Shermanii</u> EMC 1140 and EMC 1163 grown in whey permeate containing different concentrations of kefir whey at 30 C for 11 day (264 h).	88
22	Probability (PR F) parameters of GLM statistical analysis for different criteria of <u>Propionibacterium freudenreichii</u> Subsp. <u>Shermanii</u> grown in whey permeate containing different concentrations of kefir whey at 30 C for 11 day (264 h).	90
23	TSRT results obtained for the different effects of kefir whey concentrations on different average criteria of <u>Propionibacterium freudenreichii</u> Subsp. <u>Shermanii</u> EMC 1140 and EMC 1163, grown at 30 C for 11 days in whey permeate.	91

LIST OF FIGURES

No.		Page
1	Growth curve of <u>Propionibacterium freudenreichii</u> (EMC 1137, 1140, 1163) and <u>Propionibacterium jensenii</u> (EMC 1142,1143) grown in whey permeate at 30 C for 11 day(264 h)	45
2	Specific growth rate (μ) and doubling time (t_d) to <u>Propionibacterium</u> strains (EMC 1137, 1140, 1163, 1142, 1143), grown in whey permeate at 30 C for 11 day (264 h).	48
3	Production of biomass, B12, B12/biomass, utilized lactose and final BOD by <u>Propionibacterium</u> strains (EMC 1137, 1140, 1163, 1142, 1143), grown in whey permeate at 30 C for 11 day (264 h).	53
4	Effect of adding different choline chloride concentrations on Biomass, B12 production and B12/biomass ratio by <u>P.freudenreichii</u> subsp. <u>shermanii</u> (EMC 1140 and 1163), grown in whey permeate at 30 C for 11 day (264 h).	58
5	Effect of adding different Glutamic acid concentrations on biomass, B12 production and B12/biomass ratio by <u>P.freudenreichii</u> subsp. <u>sheramnii</u> EMC 1140 and 1163, grown in whey permeate at 30 C for 11 day (264 h).	65
6	Effect of adding different rape seed extract concentrations on biomass, B12 production and	

No.		Page
	B12/biomass ratio by <u>P.freudenreichii</u> subsp. <u>sheramni</u> (EMC 1140 and 1163) grown in whey permeate at 30 C for 11 day (264 h).	71
7	Effect of adding different sodium lactate concentrations on Biomass, B12 production and B12/biomass ratio by <u>P.freudenreichii</u> subsp. <u>sheramni</u> (EMC 1140 and 1163), grown in whey permeate at 30 C for 11 day (264 h).	77
8	Effect of adding different yeast extract concentrations on biomass, B12 production and B12/biomass ratio by <u>P.freudenreichii</u> subsp. <u>shermanii</u> (EMC 1140 and 1163), grown in whey permeate at 30 C for 11 day (264 h).	83
9	Effect of adding different kefir whey concentrations on biomass, B12 production and B12/biomass ratio by <u>P.freudenreichii</u> subsp. <u>shermanii</u> (EMC 1140 and 1163), grown in whey permeate at 30 C for 11 day (264 h).	89

INTRODUCTION