FACTORS AFFECTING THE RATE OF ACETIC ACID PRODUCTION BY SPECIES OF *ACETOBACTER*

by

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B. INTRODUCTION

A review was made of the literature on acetic acid bacteria. Publications were found about stock-culture media, classification and the nutritional requirements for the growth of Acetobacter species. Research had not appeared to be done, however, on either the physical conditions or the nutrients relative to an efficient production of acetic acid from ethyl alcohol. The work reported in this thesis is an attempt to provide information that heretofore has been lacking about the Acetobacter group of bacteria. Suitable methods for isolation, subculture, and classification described by other authors were adapted for the present study. Various methods were used to find an appropriate and convenient way of allowing oxidation of the alcohol-containing media to occur and to determine differences in the rates of oxidation. The best method was selected for a systematic study of the effect of varying physical and chemical factors on acid production.

C. LITERATURE REVIEW

1. History of Vinegar Production

Vinegar has been known for many years and has been used as a condiment, preservative, and medicine. It has been referred to in the Old Testament of the Bible. Vaughn (49) stated that many famous scientists of the Middle Ages and later spent much time in the study of vinegar, its characteristics, uses and improvement of manufacture.

Vaughn (49) described advancements during the 19th Century in knowledge of the role of bacteria in the biological acetification of ethyl alcohol, and the relation of the recognition that more than one species of bacteria cause the oxidation of ethyl alcohol to the search for and description of other species of acetic acid bacteria. Davy in 1820 first made acetic acid synthetically by oxidizing wine with platinum black. A few years later Boerhaave apparently first suggested the plant nature of "mother of vinegar"in the literature. Kützing demonstrated that "mother of vinegar" was composed of minute organisms arranged together in chains. He classified these organisms as algae, named them Ulvina aceti and asserted that the acetification of alcohol was the result of their activity. Liebig, the celebrated German chemist, thought that the microorganisms were incidental rather than causal. He

classed "mother of vinegar" with platinum black and defined their action as identical and entirely chemical in nature. He so dominated the chemists from 1840 to 1860 that few dared to question his conclusions in public. Pasteur's experiments, published in 1868, confirmed the living nature of "mother of vinegar". They also proved that the "mother" was the active agent in acetification. Pasteur was the first to make a systematic study of the process of vinegar manufacture. He made a distinction between the pellicle that forms on the surface of fermenting wine and that which forms on souring wine, calling the former Mycoderma vini and the latter Mycoderma aceti. He also showed that acetification could not take place in the absence of the Mycoderma aceti. Pasteur considered "mother of vinegar" to behave in the same manner as platinum black. Knieriem and Mayer in 1873 proved that the chemical oxidation of alcohol by means of platinum black could not be classified with the biological oxidation caused by "mother of vinegar". Concentrations of ethyl alcohol and incubation temperatures could be much higher when chemical oxidations were allowed to proceed. Vaughn claimed that Pasteur did not recognize the acetifying membranes as being composed of bacteria but characterized them as "mycoderme". The organisms have been referred to as a microscopic fungus (2). Cohn in 1875 was one of the first to classify the organism of the acetifying membrane

as a bacterium. Hansen in 1879 first recognized that several species of vinegar bacteria could cause beer to sour. The three species studied by Hansen are now called *Acetobacter aceti*, *Acetobacter pasteurianum* and *Acetobacter kützingianum*.

With the acceptance of the fact that acetification could be caused by more than one species of bacteria, further work involved the finding of new species. Henneberg and Beijerinck described many new species. Many species of *Acetobacter* have been described, and new species are still being isolated. *Acetobacter* species have been isolated from containers in which vinegar has been made by their action on ethyl alcohol-containing media, and especially from infected beers, wines and other alcoholic beverages.

2. The Genus Acetobacter

a. Classification of the Genus

The acetic acid bacteria have been recognized as a separate group for many years due to their peculiar physiological and biochemical properties. At present, *Acetobacter* is accepted as the correct generic designation for the acetic acid bacteria. Beijerinck suggested the name (35). Many synonyms of *Acetobacter* have been used in the past; *Bacterium*, *Mycoderma*, *Ulvina*, *Umbina*, *Termobacterium*, *Acetobacterium*, *Acetimonas* and *Bacillus* (49).

Vaughn (49) summarized families to which bacteria of the genus *Acetobacter* have been assigned and has expressed his own

opinion with respect to the family designation. Orla-Jensen placed bacteria of the genus in the family Oxydobacteriaceae, Buchanan in the family Bacteriaceae, Winslow et al in the family Nitrobacteriaceae, Kluyver and van Niel, and Rahn in the family Pseudomonadaceae, and Bergey et al in the family Acetobacteriaceae. Vaughn claimed that purely on the subject of morphological characteristics acetic acid bacteria are closely related to bacteria of the family Pseudomonadaceae. Physiologically, they resemble the genera Pseudomonas and Phytomonas of the tribe Pseudomonadeae. He described similarities between the three genera, but claimed that the genera may be differentiated on the basis of natural habitat and oxidation of ethyl alcohol and glucose. Acetobacter species grow well in acid surroundings. They produce acetic acid by the oxidation of ethyl alcohol, and ketogenic species produce 5-ketogluconic acid by the oxidation of glucose and gluconic acid. Vaughn believed that Acetobacter species should be placed within the family Pseudomonadaceae, because a separate family Acetobacteriaceae is unappropriate to a genus so closely related, both morphologically and physiologically to other well-recognized genera. Shimwell (34) accepted Vaughn's opinion, and Breed et al (5) placed bacteria of the genus Acetobacter in the family Pseudomonadaceae, and in the tribe Pseudomonadeae where the genus Pseudomonas is located.

The differentiation of the genera Pseudomonas, Phytomonas and Acetobacter on the basis of natural habitat and oxidation of ethyl alcohol and glucose may be discussed further. With regard to natural habitat, Acetobacter species grow well in acid surroundings. Stanier (40) observed that some species of Pseudomonas can produce acetic acid by the oxidation of ethyl alcohol in a medium well buffered with calcium carbonate. He claimed that if the genus Acetobacter is to be kept, it must be redefined in a manner which no longer stresses so exclusively the capacity of organisms of the genus to produce acetic acid from ethyl alcohol. He believed that acid tolerance of Acetobacter organisms may be a better differentiating characteristic for the two genera. Although Vaughn (49) claimed that ketogenic Pseudomonas species produce 2-ketogluconic acid and the ketogenic acetic acid bacteria produce 5-ketogluconic acid, he mentioned that the production of 2-ketogluconic acid by Acetobacter suboxydans has been reported. Walker and Kulka (50) worked with a strain of Acetobacter suboxydans which at first yielded gluconic acid and 5-ketogluconic acid when grown on a glucose yeast-water medium in the presence of calcium carbonate and later yielded gluconic acid and a little 2-ketogluconic acid.

Breed et al (5) gave the following description of bacteria of the genus Acetobacter. Individual cells are ellipsoidal to long and rod-shaped, occurring singly, in pairs, or in short or long chains; motile with polar flagella, or

non-motile; involution forms of cells may be spherical, elongated, filamentous, club-shaped, swollen, curved or even branched. Young cells are Gram-negative, but older cells are often Gram-variable. The organisms are obligate, aerobes and usually catalase-positive. They oxidize various organic compounds to organic acids and other oxidation products which may undergo further oxidation; common oxidation products include acetic acid from ethyl alcohol, gluconic and sometimes ketogluconic acid from glucose, dihydroxyacetone from glycerol and sorbose from sorbitol. Nutritional requirements vary from simple to complex. Development of the organisms is usually best in yeast infusion or yeast autolyzate media with added ethyl alcohol or other oxidizable substrate. Optimum temperatures for growth vary with the species. Acetobacter are widely distributed in nature. They are particularly abundant in plant materials undergoing alcoholic fermentation. Organisms of the family Pseudomonadeae do not form spores.

b. Culture Methods

Many of the media for the cultivation of Acetobacter are materials used in the brewing industry. Shimwell (37) claimed that through the influence of medical bacteriology many of the media used to study bacteria connected with brewing in the past have been products of animal tissue. There appears to be no reason for using these complex media for general cultivation

or isolation of vegetable saprophytes, except when making a complete study of cultural characteristics on a wide variety of media. Yeast and the materials available in the brewery, notably malt wort, provide media of optimum composition for most of the organisms with which brewers are concerned. Shimwell was concerned primarily with the study of beer spoilage species. He claimed that sub-cultivation in hopped beer is often the only way of avoiding the loss of beer-spoilage properties. Chalk is often added to prevent the death of acid-producing cultures by their own high acid yield. Shimwell (35) indicated that even if acetic acid bacteria were fastidious in their requirements for vitamins and growth factors, wort and beer are quite rich in such substances. Tosic (44) observed that acetic acid bacteria may be beer contaminants, and may grow well in malt, fruit extract or in their alcoholic fermentation products.

Vaughn (49) claimed that three stock culture media have been used with success. Yeast water-glucose agar prepared with 2 per cent calcium carbonate is a satisfactory medium for most cultures that cause overoxidation. Sorbitol agar containing sorbitol, yeast extract powder (Difco) and agar is also satisfactory; such a medium has an advantage over glucose media because the chief oxidation products are not acidic in nature. Liver infusion broth containing Bacto-tryptone, potassium hydrogen phosphate and infused liver

particles diluted with water has also been used.

Walker and Kulka (50) have worked extensively with Acetobacter species and have described media for the laboratory cultivation of the bacteria. They have used principally malt wort and beer as media for the maintenance of pure cultures of Acetobacter species. They claimed that the addition of ethyl alcohol to both media intensifies growth. The addition of glucose to beer greatly promotes growth of Acetobacter in beer. Although most species of acetic acid bacteria grow well in plain beer, occasionally a strain is encountered which does not; such a strain is often one which has been maintained for a long time in a laboratory. They observed that yeast water with various sugars or polyhydroxy alcohols makes useful media. Not all acetic acid bacteria are capable of growth in plain yeast water, that is, without the addition of another nutrient or other nutrients. The addition of ethyl alcohol to yeast water greatly encourages the growth of numerous Acetobacter species inoculated in it, but fails to stimulate the development of others; glycerol usually encourages growth; glucose allows the growth of practically all species of Acetobacter. Some strains of Acetobacter change, however, in their oxidizing abilities toward certain substrates after being maintained for several weeks solely on yeast water media. Walker and Kulka claimed that a medium containing yeast autolyzate and glucose is also good for subculturing Acetobacter species.

They suggested that corn steep liquor ensures provision of a full range of nutrients when added to culture media, and when added in small quantity to such media as wort and beer greatly assists in assuring maintenance of typical behaviour of Acetobacter species. The media described are liquids.

Acetobacter species cannot always be isolated from samples by direct plating methods. Shimwell (36) claimed that although beer spoilage bacteria, including Acetobacter, proliferate freely in liquid media of suitable composition, they do not as a rule readily form easily visible colonies on or in solid media. Even the small colonies they normally produce may attain visible size only after somewhat prolonged incubation. Vaughn (49) found that isolation of Acetobacter may be accomplished by direct plating or streaking if the material to be examined is heavily populated with Acetobacter. Preliminary enrichment cultures in media favourable for the growth of Acetobacter are necessary when a sample contains few acetic acid bacteria in a predominating flora of yeasts and other bacteria. Vaughn suggested yeast water-glucose agar or broth and wort agar or broth for isolation purposes. Beer, wine and fruit infusions are frequently used as basal materials for isolation media. To favour growth of Acetobacter at the expense of other organisms small amounts of acetic acid and ethyl alcohol are added. Marshall and Walkley (23) isolated Acetobacter species from apples by the direct plating of apple

pulp in various concentrations in agar medium containing apple juice and ammonium phosphate.

Acetobacter species develop at room temperature and may tolerate acid media. These facts are revealed by the type of environment in which the bacteria are usually found.

Research on the nutritive requirements for the growth of species of Acetobacter has been reported by various investigators. Species and strains vary greatly in their nutritive requirements. Much work, however, still remains to be done. Visser't Hooft partially classified Acetobacter organisms by their ability to utilize ammonium salts as sole source of nitrogen in Hoyer's medium (49). Rao and Stokes (29) observed that the ability to use ammonium nitrogen is more widespread among acetic acid bacteria than was previously thought. The ability only becomes manifest if the medium contains not only the required growth factors but also the appropriate sources of carbon. They also observed that growth factor requirements vary between strains of the same species. Walker and Kulka (50) claimed that Acetobacter species are non-proteolytic. A review of the literature (to July 1954) on the use of synthetic media for culturing Acetobacter species was made; the information obtained from that review is reported in Table I.

TABLE I

SYNTHETIC MEDIA USED FOR MAINTENANCE OF ACETOBACTER SPECIES

Investigators	Underkofler et al (48)		Lampen et al (16)	Landy and Dicken (17)	Karabinos and Dicken (14)	Gray and Tatum (10)	Sarett and Cheldelin (31)		Cheldelin and Bennett (7)	Stokes and Larsen (41)		Ackermann and Shive (1)	Kaushal and Walker (15)	Litsky et al (21)	Litsky and Goldman (22)	Tepper and Litsky (43)	Rao and Stokes (29)		Fods and Vaughn (9)			Hall et al (12)	Hainbow and Mitson (28)		Brown and Snell (6)
Organisms	A. suboxydans	A. suboxydans	A. suboxydans	A. suboxydans	A. suboxydans	A. melanogenum	A. suboxydans		A. suboxydans	A. suboxydans	A. suboxydans	A. suboxydans	A. acetigenum A. pasteurianum A. kützingianum	A. xylinum	A. xylinum	A. xylinum	A. xylinum A. rancens A. suboxydans A. gluconicum A. melanogenum		A. melanogenum	A. oxydans	A. rancens	Acetobacter 10 species [c]	A. acidum-mucosum A. mobile A. suboxydans	A. capsulatum A. gluconicum A. turbidans A. viscosum	A. suboxydans
Remarks	Poor growth	Normal growth	Best growth for testing p-amino-benzoic acid	Best growth for testing p-amino-benzoic acid	1/2 Maximum growth for testing nicotinic acid	Good growth	Best growth for the smallest amount of pantothenic acid [a]		Best growth for smallest amount of p-amino-benzoic acid	Growth in 20 amino acids similar to hydrolyzed casein	Same growth as with 20 amino acids	Growth with pantothenic acid and related compounds	Cellulose production	Acid production greater with adenine, guanine, uracil	Organism could synthesize most Vitamin B's	Growth in medium containing Casamino acids or 20 amino acids	10 Vitamins do not support growth of all strains; require yeast extract		Minimum requirements for growth			No growth with 1 species	Growth with casein hydrolysate as nitrogen source		Good growth
Concentrations per 100 ml.												See Sarett and Cheldelin													
Carbon:																									
1.Ethanol								1.					2.5 gm.					1.							
2.Glucose							0.25 gm.	2.	0.25 gm.				2.0 gm.	3.0 gm.	3.0 gm.	3.0 gm.	2.0 gm. (or ethanol)	2.	1.0 gm.	1.0 gm.	1.0 gm.	2.6 gm.	2.0 gm.	2.0 gm.	
3.Glycerol	5.0 gm.	5.0 gm.	5.0 gm.	5.0 gm.	5.0 gm.	5.0 gm.	5.0 gm.	3.	5.0 gm.	5.0 gm.	5.0 gm.							3.							5.0 gm.
4.Lactic acid (AR)								4.										4.					1.0 gm.		
Salts:																									
1.Ammonium sulphate						1.0 gm.		1.					0.3 gm.				1.0 gm.[b]	1.	1.0 gm.	1.0 gm.	1.0 gm.		0.1 gm.	0.1 gm.	
2.Ferrous sulphate heptahydrate	0.001 gm.	0.001 gm.	0.001 gm.	0.001 gm.	0.001 gm.	0.001 gm. ($-H_2O$)	0.001 gm.	2.	0.001 gm.	0.001 gm.	0.001 gm.			0.001 gm.	0.001 gm.	0.001 gm.	0.001 gm.	2.	0.001 gm.	0.001 gm.	0.001 gm.				0.001 gm.
3.Magnesium sulphate heptahydrate	0.02 gm.	0.02 gm.	0.02 gm.	0.02 gm.	0.02 gm.	0.02 gm.	0.02 gm.	3.	0.02 gm.	0.02 gm.	0.02 gm.		0.2 gm.	0.02 gm.	0.02 gm.	0.02 gm.	0.02 gm.	3.	0.02 gm.	0.02 gm.	0.02 gm.		0.2 gm.	0.2 gm.	0.02 gm.
4.Manganese sulphate tetrahydrate	0.001 gm. ($3\ H_2O$)	0.001 gm. ($3\ H_2O$)	0.001 gm. ($3\ H_2O$)	0.001 gm. ($2\ H_2O$)	0.001 gm. ($3\ H_2O$)	0.001 gm. ($-H_2O$)	0.001 gm.	4.	0.001 gm.	0.001 gm. ($3\ H_2O$)	0.001 gm. ($3\ H_2O$)			0.001 gm. ($3\ H_2O$)	0.001 gm. ($3\ H_2O$)	0.001 gm. ($3\ H_2O$)	0.001 gm.	4.	0.001 gm.	0.001 gm.	0.001 gm.				0.001 gm. ($3\ H_2O$)

a - Purines and pyrimidines necessary.
b - If no casein hydrolysate.
c - Growth with A. acetobacter pasteurianum, A. acidum-mucosum, A. ascendens, A. capsulatum, A. melanogenum, A. rancens, A. suboxydans, A. viscosum, A. acetosum. No growth with A. gluconicum.

continued

TABLE I continued

Investigators	Underkofler et al (48)		Lampen et al (16)	Landy and Dicken (17)	Karabinos and Dicken (14)	Gray and Tatum (10)	Sarett and Cheldelin (31)		Cheldelin and Bennett (7)	Stokes and Larsen (41)		Ackermann and Shive (1)	Kaushal and Walker (15)	Litsky et al (21)	Litsky and Goldman (22)	Tepper and Litsky (43)	Rao and Stokes (29)		Foda and Vaughn (9)			Hall et al (12)	Rainbow and Mitson (28)		Brown and Snell (6)
Salts: continued																									
5. Potassium dihydrogen phosphate	0.05 gm.	0.05 gm.	0.05 gm.	0.05 gm.	0.05 gm.	0.05 gm.	0.05 gm.	5.	0.05 gm.	0.05 gm.	0.05 gm.		0.3 gm.	0.05 gm.	0.05 gm.	0.05 gm.	0.05 gm.	5.	0.05 gm.	0.05 gm.	0.05 gm.		0.3 gm.	0.3 gm.	0.05 gm.
6. Potassium hydrogen phosphate	0.05 gm.	0.05 gm.	0.05 gm.	0.05 gm.	0.05 gm.	0.05 gm.	0.05 gm.	6.	0.05 gm.	0.05 gm.	0.05 gm.			0.05 gm.	0.05 gm.	0.05 gm.	0.05 gm.	6.	0.05 gm. ($3\ H_2O$)	0.05 gm. ($3\ H_2O$)	0.05 gm. ($3\ H_2O$)				0.05 gm.
7. Sodium chloride	0.001 gm.	0.001 gm.	0.001 gm.	0.001 gm.	0.001 gm.	0.001 gm.	0.001 gm.	7.	0.001 gm.	0.001 gm.	0.001 gm.			0.001 gm.	0.001 gm.	0.001 gm.	0.001 gm.	7.	0.001 gm.	0.001 gm.	0.001 gm.				0.001 gm.
8. Trace element solution								8.										8.					0.05 ml.	0.05 ml.	
Amino acids and growth factors:																									
General																									
1. Liver concentrate (norit A. treated)							0.1 gm.	1.	0.1 gm.									1.							
2. Liver extract (soluble)	✓[d]							2.										2.							
3. Yeast extract								3.										3.							20.0 mg.
Amino acids:																									
1. Casein	0.3 - 0.5 gm. (hydrolyzed)	0.3 - 0.5 gm. (hydrolyzed)	0.3 - 0.5 gm. (- p-ABA)[e]	0.3 gm. (hydrolyzed)	0.3 gm. (hydrolyzed)		0.5 gm. (hydrolyzed)	1.	0.5 gm. (hydrolyzed)	0.3 - 0.5 gm. (hydrolyzed)				0.5 gm. (Cas.a.a.)[g]	0.5 gm. (Cas.a.a.)	0.5 gm. (Cas.a.a.)	0.5 gm. (hydrolysate)	1.	0.5 gm.[h]	0.5 gm.[h]	0.5 gm.[h]		0.6 gm. (hydrolyzed)	0.6 gm. (hydrolyzed)	0.3 - 0.5 gm. (hydrolyzed)
2. Peptone (norit A. treated)							0.25 gm.	2.	0.25 gm.									2.							
3. Alanine								3.		20.0 mg.	20.0 mg.					20.0 mg.		3.		20.0 mg.	20.0 mg.	66.7 mg.(dl)			
4. B-Alanine							0.1 mg.	4.								20.0 mg.		4.							
5. Phenylalanine								5.		20.0 mg.						20.0 mg.		5.				66.7 mg.(dl)			
6. Arginine								6.		20.0 mg.						20.0 mg.		6.				66.7 mg.			
7. Asparagine	✓						2.0 mg.[f]	7.								20.0 mg.		7.				66.7 mg.			

d - ✓ - Present, amount not indicated.
e - p-ABA - p-aminobenzoic acid.
f - Curtails growth with pantothenic acid.
g - Cas.a.a. - Casamino acids.
h - Hydrolyzed casein if testing growth factors.

continued

TABLE I continued

Investigators	Underkofler et al (48)		Lampen et al (16)	Landy and Dicken (17)	Karrabinos and Dicken (14)	Grey and Tatum (10)	Sarett and Cheldelin (31)
Amino acids: continued							
8. Aspartic acid							
9. Cysteine							
10. Cystine	7.5 mg.	7.5 mg.	7.5 mg.	7.5 mg.	7.5 mg.		7.5 mg.
11. Glutamic acid							
12. Ptercylglutamic acid							
13. Glutamine	≠						
14. Glutathione	≠						
15. Glycine							
16. Histidine							
17. Leucine							
18. Isoleucine							
19. Norleucine							
20. Lysine							
21. Methionine							
22. Proline						0.02 gm.	
23. Hydroxyproline							
24. Serine							
25. Taurine							2.0 mg.[i]
26. Threonine							
27. Tryptophane	10.0 mg.	10.0 mg.	10.0 mg.	10.0 mg.	10.0 mg.		10.0 mg.
28. Tyrosine							
29. Valine							
30. Norvaline							

	Cheldelin and Bennett (7)	Stokes and Larsen (41)		Ackermann and Shive (1)	Kaushal and Walker (15)	Litsky et al (21)	Litsky and Goldman (22)	Tepper and Litsky (43)	Rao and Stokes (29)
8.		20.0 mg.						20.0 mg.	
9.								20.0 mg.	10.0 mg. (with cas. h)[j]
10.	7.5 mg.	20.0 mg.	20.0 mg.						
11.		20.0 mg.						20.0 mg.	
12.									1.0 µg.
13.		[illegible]						[illegible]	
14.									
15.		20.0 mg.	[illegible]					20.0 mg.	
16.		20.0 mg.	20.0 mg.					20.0 mg.	
17.		20.0 mg.	[illegible]					20.0 mg.	
18.		20.0 mg.	20.0 mg.					20.0 mg.	
19.		20.0 mg.						[illegible]	
20.		20.0 mg.						20.0 mg.	
21.		20.0 mg.						20.0 mg.	
22.		20.0 mg.	20.0 mg.					20.0 mg.	
23.		20.0 mg.							
24.		20.0 mg.						20.0 mg.	
25.									
26.		20.0 mg.						20.0 mg.	
27.	10.0 mg.	20.0 mg.						20.0 mg.	20.0 mg. (DL) (with cas. h)[j]
28.		20.0 mg.						20.0 mg.	
29.		20.0 mg.	20.0 mg.					20.0 mg.	
30.									

	Foda and Vaughn (9)			Hall et al (12)	Rainbow and Mitson (28)		Brown and Snell (6)
8.			20.0 mg. (or glutamic acid)				
9.				66.7 mg			
10.		20.0 mg.	20.0 mg.				7.5 mg.
11.				66.7 mg.			
12.							
13.							
14.							
15.				66.7 mg.			
16.		20.0 mg.	20.0 mg.	66.7 mg.			
17.				66.7 mg.			
18.		20.0 mg.	20.0 mg.	66.7 mg.(dl)			
19.				66.7 mg.(dl)			
20.				66.7 mg.(dl)			
21.				66.7 mg.(dl)			
22.		20.0 mg.	20.0 mg.	66.7 mg.			
23.				66.7 mg.			
24.				66.7 mg.(dl)			
25.							
26.				66.7 mg.(dl)			
27.				66.7 mg.(dl)			10.0 mg.
28.				66.7 mg.			
29.		20.0 mg.	20.0 mg.	66.7 mg.(dl)			
30.				66.7 mg.(dl)			

i - Inhibits growth effect of pantothenic acid.
j - Cas. h - Casein hydrolysate.

continued

TABLE I continued

Investigators	Underkofler et al (48)		Lampen et al (16)	Landy and Dicken (17)	Karabinos and Dicken (14)	Gray and Tatum (10)	Sarett and Cheldelin (31)
Purine and pyrimidine bases:							
1. Adenine	≠						0.5 mg.
2. Guanine							0.5 mg.
3. Xanthine							
4. Uracil	≠						0.5 mg.
Vitamins:							
1. p-Aminobenzoic acid		1.0 µg.	0.5 µg.	1.0 µg.	1.0 µg.	10.0 µg.	10.0 µg.
2. B_{12}							
3. Biotin	≠	0.5 µg. (with nicotinic acid)					
4. Choline chloride							
5. Folic acid							
6. Inositol	≠						
7. Niacin amide							
8. Nicotinic acid	≠		100.0 µg.	100.0 µg.	5.5 µg.	100.0 µg.	10.0 µg.
9. Pantothenic acid	≠	100.0 µg. (Ca salt)	100.0 µg. (Ca salt)	100.0 µg. (Ca salt)	100.0 µg. (Ca salt)		
10. Pantoyl l-lactone						100.0 µg.	
11. Pyridoxal·HCl							
12. Pyridoxamine·2HCl							
13. Pyridoxine	≠						
14. Riboflavin	≠						
15. Thiamine	≠					100.0 µg.	
16. Pimelic acid	≠						

	Cheldelin and Bennett (7)	Stokes and Larsen (41)		Ackermann and Shive (1)	Kaushal and Walker (15)	Litsky et al (21)	Litsky and Goldman (22)	Tepper and Litsky (43)	Rao and Stokes (29)
1.	0.5 mg.					0.002 gm. (sulphate)	0.002 gm. (sulphate)	0.002 gm. (sulphate)	
2.	0.5 mg.					0.002 gm.	0.002 gm.	0.002 gm.	
3.									
4.	0.5 mg.					0.002 gm.	0.002 gm.	0.002 gm.	
1.	0.1 µg.	1.0 µg.	1.0 µg.			20.0 µg.	20.0 µg.	20.0 µg.	10.0 µg.
2.									0.1 µg.
3.		0.5 µg. (with nicotinic acid)	0.5 µg. (with nicotinic acid)			0.5 µg.			0.04 µg.
4.									
5.						20.0 µg.			
6.						20.0 µg.			0.5 mg.
7.						20.0 µg.			
8.	10.0 µg.								40.0 µg.
9.	20.0 µg. (Ca salt)	100.0 µg. (Ca salt)	100.0 µg. (Ca salt)			20.0 µg. (Ca salt)			40.0 µg.
10.									
11.									
12.									
13.						40.0 µg.			80.0 µg.
14.						40.0 µg.			40.0 µg.
15.						20.0 µg.			40.0 µg.
16.									

	Foda and Vaughn (9)			Hall et al (12)	Rainbow and Mitson (28)		Brown and Snell (6)
1.				1.38 mg. (sulphate·$2H_2O$)		1.0 mg.	
2.				1.3 mg. ($2H_2O$)			
3.				1.2 mg.			
4.				1.2 mg.			
1.	20.0 µg.	20.0 µg.	20.0 µg.	0.01 mg.	10.0 µg.	10.0 µg.	
2.							
3.				0.0005 mg.	0.08 µg. (D)	0.08 µg. (D)	
4.				1.0 mg.			
5.				0.0005 mg.			
6.				2.5 mg.			
7.							
8.	20.0 µg.	20.0 µg.	20.0 µg.	0.2 mg.	100.0 µg.	100.0 µg.	
9.	100.0 µg.	100.0 µg.	100.0 µg.	0.2 mg. (dl Ca salt)	100.0 µg. (Ca D salt)	100.0 µg. (Ca D salt)	5.0 µg. (Ca salt)
10.							
11.				0.01 mg.			
12.				0.01 mg.			
13.				0.16 mg.	100.0 µg.	100.0 µg.	
14.				0.2 mg.	10.0 µg.	10.0 µg.	
15.	100.0 µg.		100.0 µg.	0.1 mg.	100.0 µg.	100.0 µg.	
16.							

c. Classification of Species

A great many species of Acetobacter have been described. Many are no longer recognizable, others are possibly synonyms for previously described species.

Henneberg in his classification of acetic acid bacteria in 1926 recognized distinct species as being associated with wort, beer, wine and generator vinegar (49). His influence has been noticeable in most classifications of Acetobacter, but his classification is undesirable. He listed one of the common species Acetobacter aceti with beer vinegar bacteria. Vaughn (49) isolated the same organism from grape juice, unfinished wine, souring figs and dates, dried fruits and vinegar, as well as from sour beer. Walker and Kulka (50) said that some microorganisms find in certain natural media the particular nutrients which best promote their growth and, having gained access as chance infections, establish themselves in these media. Thus in time artificially modified types, particularly adapted to growth in such media, develop. Generally speaking, the behaviour of an organism is conditioned by its previous history.

Vaughn (49) claimed that if acetic acid bacteria are differentiated from the "utilitarian point of view", they must be divided into two groups, depending upon their ability to oxidize a substrate such as alcohol or glucose. Those bacteria causing incomplete oxidations are important for the preparation of pharmaceuticals. Since many species have

been described and named, some of which are probably synonyms for previously described species, Vaughn includes a "key" to differentiate well-recognized species. The "key", which seems to be similar to that of Visser't Hooft, has proved satisfactory for the differentiation of most of the cultures. Vaughn stressed that an intensive study of the genus may require expansion of the "key" to differentiate all species adequately. He said that the scheme for classification of the species of Acetobacter was patterned after that of the Europeans, particularly the Dutch, since Americans have not been greatly interested in the genus until recently. He believed that biochemical studies of species of Acetobacter had far excelled taxonomic advances. The "key" is as follows:

I "Oxidize acetic acid to CO_2 + H_2O
- A. Utilize ammonium salts as a sole source of nitrogen (Hoyer's medium): Acetobacter aceti (Kützing) Beijerinck
- B. Do not utilize ammonium salts as a sole source of nitrogen
  1. Forms a thick, cellulosic membrane on the surface of liquids: Acetobacter xylinum (Brown) Bergey et al
  2. No cellulosic membrane formed: Acetobacter rancens Beijerinck

II Do not oxidize acetic acid
- A. Form pigments in glucose media
  1. Dark brown pigment: Acetobacter melanogenum Beijerinck
  2. Pink to rose pigment: Acetobacter roseum (Takahashi and Asai) nov. comb.
- B. Do not form such pigments
  1. Optimum temperature 30° to 35° C: Acetobacter suboxydans Kluyver and de Leeuw
  2. Optimum temperature 20° C: Acetobacter oxydans Henneberg."

Vaughn differentiated Acetobacter species primarily on their ability to oxidize acetic acid to carbon dioxide and water. Other bacterial species can also oxidize acetic acid in a similar way. For example, Oginsky and Umbreit (24) maintained that the tricarboxylic acid cycle exists in some bacterial species. The products formed during the oxidation of acetate by the cycle are carbon dioxide and water. Species other than those of Acetobacter can oxidize acetate by the cycle. Swim and Krampitz (42) presented evidence for the oxidation of acetate by the tricarboxylic acid cycle in Escherichia coli, and Saz and Krampitz (32) in Micrococcus lysodeikticus. As presented the classification described by Vaughn does not recognize many of the other named species; he included some as strains of the species mentioned in the scheme. He did not investigate the status of Acetobacter gluconicum, Acetobacter dihydroxyacetonicum, Acetobacter capsulatum, Acetobacter viscosum and others.

Shimwell (35) claimed that since Acetobacter show wide variation in the number of carbohydrates and alcohols they attack, certain workers have been attracted by this criterion as a basis for the differentiation of species. For example, Henneberg arranged Acetobacter species in accordance with the number of carbohydrates they acidify. Shimwell asserted that until an extensive study of a larger number of strains has been undertaken, acid production from different sugars and alcohols probably cannot be depended upon as reliable

criterion for the differentiation of individual Acetobacter species, although broad types may perhaps be established on the basis of acid production. Shimwell included a "key" based on that of Vaughn (49) in which there were formerly undescribed beer-spoilage species. He emphasized that species differentiation is almost always subjective, but never more than with the genus Acetobacter; each new attempt to utilize fresh criteria for the differentiation has tended to demolish the validity of the criteria used by previous workers without necessarily leading to a better system. Shimwell believed that Henneberg's original and extensive work on the acetic acid bacteria was at that time the most authoritative, accurate and detailed account of many industrially important Acetobacter species.

Tosic and Walker (46 and 47) claimed that considerable difficulty was experienced in identifying various species of acetic acid bacteria isolated. The description of Acetobacter species appearing in the literature, particularly early reports, were lacking in many details which today are recognized as essential for correct comparison. They elaborated standard culture media and procedures suitable for the characterization of ten authentic cultures of named species of Acetobacter from the National Collection of Type Cultures, the Lister Institute, Elstree, England. The investigation of morphological characteristics included the shape and size of cells in hanging drop preparations and on stained slides, arrangement of cells,

involution forms, motility and capsule formation. Cultural characteristics included time at which growth first became noticeable and the description of single colonies. Biochemical characteristics included gas production in standard malt wort and in glucose yeast water, acid production in yeast water medium containing various carbon sources, utilization of free acetic acid, the Voges-Proskauer reaction and the catalase test. Physiological characteristics included temperature for maximum growth, aerobiosis, pH, and tolerance for ethyl alcohol. They claimed that in the majority of instances the description of characteristics were in good agreement with those previously published. They claimed that the technique described represents the first attempt to standardize the procedure for the characterization of members of the genus.

Walker and Kulka (50) have summarized the importance of determining certain morphological, cultural, physiological and biochemical characteristics in identifying Acetobacter species. Morphological characteristics, with the exception of motility, are not of great value in classification since many species have similar cells. Cultural characteristics are of considerable assistance, the most useful evidence probably being the type of growth in wort and beer, the behaviour and appearance of streak cultures on wort agar, and the type of giant colony produced on semi-solid wort-agar. The most important physiological

characteristics are incubation temperature and oxygen requirement of cultures. Biochemical characteristics such as acid production from carbohydrates, ability to develop in the presence of ammonium salts as sole source of nitrogen, and ketogenic activity are useful. They claimed that species differentiation cannot be discussed without some reference to variability of behaviour. Cultures may lose the power of pellicle formation and produce instead a faint ascending film. Later the cultures may regain the ability to produce a tough, cohesive pellicle. Instances are known of an organism acquiring the power to produce acid from a sugar which normally it cannot utilize, also of an organism to lose its ability to form acid from a particular carbohydrate. The same is true of ketogenic activity. Certain species can metabolize glucose in media such as those of Henneberg and of Hoyer in which ammonium salts are the sole source of nitrogen; occasionally an Acetobacter species will grow in one or both media when first isolated from its natural habitat, only to lose such ability irretrievably after some time in artificial cultivation. Walker and Kulka have worked with strains of Acetobacter aceti which cannot utilize ammonium salts as sole source of nitrogen. Most workers in general believe Acetobacter aceti can utilize ammonium salts as sole source of nitrogen.

It appears that more attention must be given to the classification of Acetobacter species. As mentioned in the

preceding paragraph many difficulties occur in classification due to variability in behaviour of the organisms. Breed et al (5) used Vaughn's "key", see page 17, to identify Acetobacter species. They stated, however, that species related to Acetobacter rancens may be able to use inorganic salts as sole source of nitrogen.

3. Vinegar Production

The oxidations of certain substrates brought about by various species of the genus Acetobacter are of particular significance because some industrially important compounds formed, such as ketoses and keto acids, are prepared with considerable difficulty by purely chemical methods. Species of the genus vary in their ability to oxidize or dehydrogenate various substrates. Some species almost completely oxidize a substrate, sometimes forming carbon dioxide and water as the principal end products. These bacteria apparently have no industrial value. Other species of the genus bring about the incomplete oxidation of a substrate and, accordingly, may be of much importance. Acetobacter suboxydans is a species well-adapted for industrial use for it generally brings about the incomplete oxidation of sugars, alcohols and acids even when a liberal supply of oxygen is available, which is essential for a rapid dehydrogenation of the substrate. Acetobacter species in general oxidize ethyl alcohol to acetic acid and d-sorbitol to l-sorbose.

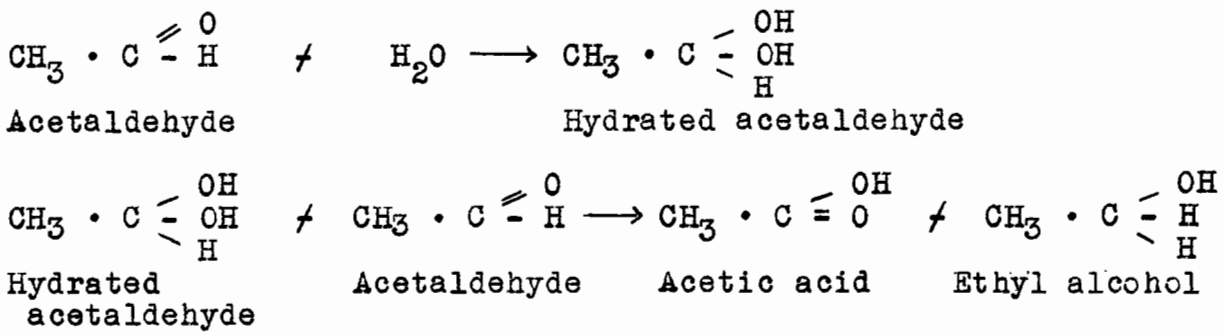
Certain species oxidize glycerol to dihydroxyacetone. Some species have also been used in the making of perseulose from perseitol (an alcohol that occurs as a constituent of the avocado); gluconic acid and 5-ketogluconic acid from glucose; acetylmethylcarbinol from meso- and levorotatory-2, 3-butandiol; l-erythrulose from meso-erythritol; and d-tartaric acid from glucose. Vaughn (49) claimed that from the economic standpoint, the greatest importance of the acetic acid bacteria is in the manufacture of vinegar.

a. Mechanism of Fermentation

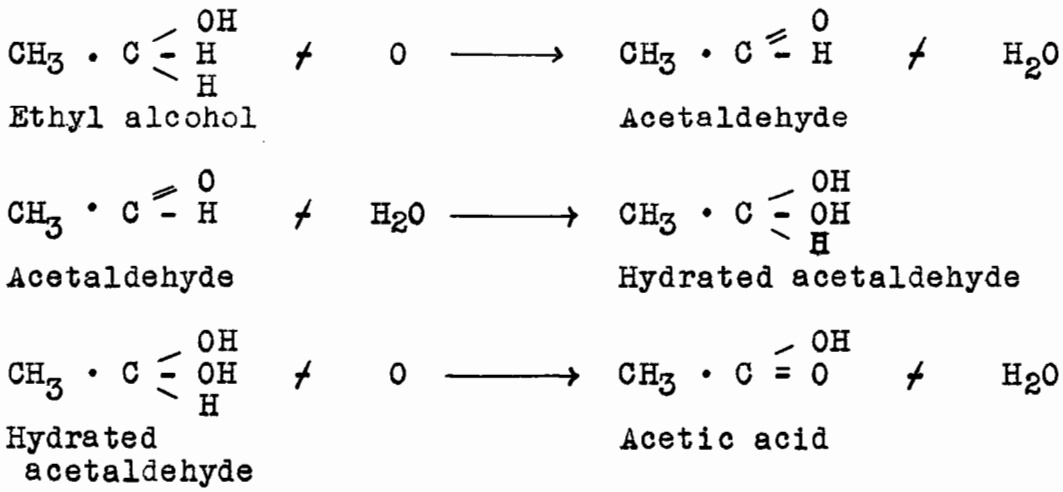
Various 19th Century research workers observed pertinent facts concerning the oxidation of ethyl alcohol by platinum black (49). The present explanation of the oxidation of ethyl alcohol by Acetobacter species involves these facts. Davy first made acetic acid synthetically by oxidizing wine with platinum black. Döbereiner found that during the oxidation, the alcohol absorbs oxygen with the formation of acetic acid and water. Liebig studied an intermediate compound formed during oxidation and named it "aldehyde".

Several ideas have been expressed to explain the mechanism of fermentation by Acetobacter species (27). One explanation of the mechanism is that Acetobacter species aerobically transform ethyl alcohol to acetaldehyde, and in turn dismute acetaldehyde to equimolar quantities of ethyl

alcohol and acetic acid:



Alternate oxidation and dismutation occur until Acetobacter species convert all the ethyl alcohol to acetic acid. As examples for the reasoning of the mechanism, Acetobacter ascendens, Acetobacter pasteurianum, and Acetobacter xylinum can dismute acetaldehyde to equimolar quantities of acetic acid and ethyl alcohol anaerobically. Another explanation of the mechanism is that Acetobacter species dehydrogenate ethyl alcohol to acetaldehyde and hydrated acetaldehyde to acetic acid. Oxygen is the hydrogen acceptor:



When conditions become favourable Acetobacter species may convert acetaldehyde to acetic acid by dismutation.

Acetobacter enzymes induce the oxidation of alcohol to acetaldehyde and of acetaldehyde to acetic acid (49).

b. Methods of Manufacture

Vinegar is the condiment made from sugary or starchy materials by alcoholic and subsequent acetous fermentations. The term signifies "sour wine" according to its derivation from the French. The composition of a vinegar will depend somewhat on the nature of the raw material that has undergone alcoholic and acetous fermentations. The conditions of manufacture, aging and storage will also influence the composition of the product. The Food and Drug Administration of the United States[/] in 1936 defined vinegar as containing at least 4 gm. of acetic acid per 100 ml. vinegar at 20° C., as well as small amounts of alcohol, glycerin, esters, reducing substances, salts and other compounds.

Vinegar may be manufactured from a wide variety of raw materials, the main requirement being a satisfactory economic source of alcohol. Apple vinegar, or cider vinegar, is commonly used in the United States; wine vinegar is extensively used in the large grape-growing areas of Europe, and malt vinegar in England. In food industries, where vinegar serves both as a condiment and a preservative, large quantities of

/ - U.S. Dept. of Agriculture, F.D.A., Service and Regulatory Announcements, Food and Drug, No. 2, Rev. 5, November, 1936, Cited by Prescott and Dunn (27).

spirit, distilled, or grain vinegar are consumed. Surplus products, such as fresh beets, pears, peaches, plums, figs, oranges, pineapples and berries, can be used for the production of vinegar. Surplus dried fruits, including prunes, apples, figs, peaches, apricots and dates, make good condiment vinegars. Honey vinegar is known and appreciated as a condiment. Hydrolyzed starch materials including potatoes, corn and rice may also be used. Sugar vinegar is made from sugar syrup, molasses, or refiners' syrup. Glucose vinegar is made from glucose solutions. Whatever is used, the raw material must first undergo alcoholic fermentation before acetification can proceed. The quality of the vinegar depends on the quality of the raw materials used. Alcohol in a concentration of 10 to 13 per cent is readily fermented.

Although there are a large number of bacteria that possess the ability to produce acetic acid in small amounts from various substrates, only relatively few possess the characteristics desired for vinegar manufacture. Different species of Acetobacter are generally used in different processes of vinegar manufacture. Usually the alcohol-containing medium is acidified with vinegar in order to inhibit the development of undesirable types of bacteria and to supply the medium with desirable acetic-acid-producing bacteria for purposes of inoculation. Heavily inoculated starters of a desirable species of Acetobacter may be used to control the bacterial population.

Since the conversion of ethyl alcohol to acetic acid is primarily an oxidative process or a dehydrogenation process in which atmospheric oxygen acts as the hydrogen acceptor, the success of the fermentation depends largely on the availability of large quantities of oxygen during the process. The use of too low a temperature, 12° to 15°C., during the fermentation process favours a slow fermentation; the use of too high a temperature, 42° to 45°C., causes Acetobacter organisms to lose their ability to function normally. The exact temperature used depends on the organism and process.

Vinegar is made either by the slow acetification of vinegar stock in barrels or by rapid acetification in generators. Both processes have been used for many years (49). The slow process, essentially the "Orleans" method, was used extensively in France for a long time before the rudimentary generator process was developed about 1670. Although Boerhaave, in 1732, has been credited by many with construction of the first vinegar generator, an anonymous description of a vinegar generator was published in 1670. The generator process became important in the early part of the 19th Century primarily because of the efforts of Schüzenbach.

i." Slow" Process

Of the slow processes the "Orleans" or "French" method is the oldest and also the best for the production of table vinegar (27). Barrels of approximately 200 l. capacity are used as the containers in the process. Each barrel is filled about one-third full with a good grade of vinegar, which constitutes the starter or culture, and 10 to 15 l. of wine is added. At weekly intervals for four weeks, the same amount of wine is added to the barrel. When five weeks have passed, 10 to 15 l. of vinegar is withdrawn from the barrel, which is now about one-half filled, and the same amount of wine is introduced. The operation may be repeated, the process thus becoming continuous. Air is admitted to the barrels through holes, one usually at each end of the barrel, which is placed on its side, above the level of the vinegar. Air may also be admitted through a top bung-hole. All holes are screened to prevent the entrance of insects. The acetic acid bacteria form a thin film on the surface of the solution. The films are easily disturbed by the addition of the alcoholic medium and the withdrawal of vinegar; if they sink, they use up the nutrient substances but fail to produce acetic acid under anaerobic conditions. Pasteur suggested the use of a support for the film.

ii. "Quick" Process

The "quick" vinegar process has also been called the "generator" or "German" process (49). The process came into industrial prominence during the early part of the 19th Century and is now in universal use for the production of most commercial vinegar. The vinegar generator is designed to afford the maximum surface exposure for a volume of vinegar stock in order to supply an optimum amount of air for the vinegar bacteria. Maximum surface exposure is accomplished by droplet dispersion of the inoculated stock as applied to the upper surface of a mass of percolating medium, beechwood shavings of some depth. The vinegar stock is allowed to trickle slowly through the generator packing. The bacteria adhere to the shavings or other packing material and find conditions optimum for rapid acetification of the alcohol contained in the stock or mix. The oxidation of alcohol takes place simultaneously throughout most of the exposed surface of the percolating medium.

The generator consists of a large, cylindrical to slightly conical-shaped tank, divided into three compartments separated by perforated wood covers. The upper compartment contains the apparatus for even distribution of the vinegar stock over the percolating medium. In operation, the vinegar stock is distributed over the percolating medium intermittently in small amounts by automatic tipping troughs or revolving spargers. The central

compartment contains the percolating medium, which may be beechwood shavings, coke, wood charcoal, basket work made of rattan, bundled rattan, excelsior, corn cobs, grape stems, pressed pomace, or other materials that offer large surface areas. The lower chamber serves for the collection of the vinegar, and contains regulating ports for the admission of air. The vinegar stock may be trickled through the same generator until the desired acetification takes place, or it may be passed through two or three or more generators connected in series.

The fermentation should be allowed to proceed until the vinegar has reached its maximum strength. The vinegar is then stored anaerobically, to prevent Acetobacter species from destroying the vinegar by oxidation; it is later clarified, bottled and Pasteurized (27). Aging takes place during storage and may require a year or longer. Aging is a process that improves the flavour and clarity of vinegar, especially vinegars made from wine and fruit juices. Esters formed during aging cause the disappearance of the harsh flavour and odour. Some vinegars may be bottled without further treatment, but most of them should be clarified first. Clarification may be effected by filtration or by fining. In the process of fining, a substance such as fish isinglass, bentonite, or Spanish clay is thoroughly mixed with the vinegar. The mixture is permitted to stand until a clear vinegar appears. The clarified vinegar is drawn or siphoned off. Bottles should

be completely filled and tightly capped. Pasteurization is done by heating the bottles to a temperature of 60° to 66°C. for about thirty minutes. The finishing procedure is the same in the "slow" process.

c. Factors Affecting Vinegar Production

The production of vinegar by Acetobacter species appears to have received more interest industrially than academically. A minor number of species have been intensely studied. Acetobacter species did not appear to have been investigated with regard to the various factors, physical and chemical, that would affect the rate of acid production at the time when the author began the investigation. Vinegar has been made biologically for many years, and the process possibly has been taken for granted. The conditions necessary for its manufacture from alcoholic solutions were known empirically long before the function of living organisms in the process was recognized (49). Probably many of the discoveries credited to others were technical secrets of the vinegar manufacturers long before scientific investigations were recorded in the literature.

i. Medium

As stated previously vinegar may be manufactured from almost any product capable of yielding alcohol by fermentation. Fruits, honey, sugar-containing syrups, hydrolyzed starchy materials, beer and wine may serve as raw materials. Shimwell (35) said that in the manufacture of spirit - vinegar industrial alcohol diluted to a suitable concentration may be used, and nutrients added to supply the nitrogen requirements of the bacteria. Cosbie, Tosic and Walker (8) observed that the oxidation of alcohol to acetic acid by Acetobacter turbidans varies directly as the concentration of the yeast water in the media; therefore to a certain limit the concentration of nutrients in the medium affects the amount of acid produced. Walker and Tosic (51) observed that Acetobacter viscosum forms acetic acid from ethyl alcohol in standard malt wort but not in plain yeast water; and Acetobacter aceti forms acetic acid from ethyl alcohol in standard malt wort and in yeast water. Walker and Kulka (50) observed that a medium consisting of ethyl alcohol and yeast water encourages the growth of Acetobacter species but not that of others. Rao and Stokes (30) found that with Acetobacter suboxydans and Acetobacter melanogenum the stimulatory activity of the biological materials for ethyl alcohol utilization is due to the sugars and related substances that they contain. They stated that apparently acetic acid bacteria are dependent upon carbohydrates for growth initiation, and that once this

has occurred, the bacteria use ethyl alcohol as a source of carbon and energy, and oxidize ethyl alcohol to acetic acid. They worked with media containing casein hydrolysate, minerals and B vitamins. Litsky and Esselin (20) observed that with Acetobacter xylinum p-aminobenzoic acid is essential, and riboflavin and pantothenic acid together are stimulatory to acid production. Other members of the B-complex group of vitamins have no effect or are inhibitory to growth.

The ethyl alcohol content of the medium is important in determining whether Acetobacter species produce acetic acid from ethyl alcohol. Although some authors claim that alcohol in a concentration of 10 to 13 per cent is readily fermented, the general observation was that Acetobacter species vary in their ability to utilize different concentrations of ethyl alcohol. Vaughn (49) claimed that concentrations of alcohol between 14 and 15 per cent by volume are very inhibitory to acetic acid bacteria, but concentrations of 5 to 12 per cent are not inhibitory. Walker and Tosic (52) asserted that an ethyl alcohol content in media greater than 4 per cent prolongs the stationary and lag phases of growth of the bacteria, but an alcohol content of more than 6 per cent inhibits growth of some species. Prescott and Dunn (27) noted that when alcohol concentrations of 14 per cent or greater are used, the zoogloeal mat forms with difficulty and Acetobacter species incompletely oxidize alcohol to acetic acid; when alcohol concentrations of less than 1 or 2 per cent are used

acetic acid may be lost, for Acetobacter species oxidize acetic acid to carbon dioxide and water.

The effect of the pH of an alcohol-containing medium on the amount of acetic acid produced does not seem to have been studied by past workers. The general observation was that Acetobacter species favour acid surroundings for growth. Shimwell (33) claimed that many acetic acid bacteria have a pH range extending well below 3.5 for growth. It appears that in a natural environment oxygen is the hydrogen acceptor in the conversion of ethyl alcohol to acetic acid; and that under anaerobic conditions, especially at high pH values (7.6 to 8.4), and to some extent in an aerobic environment, acetaldehyde may act as the hydrogen acceptor for acetaldehyde hydrate (26). The result is a dismutative change or Cannizzaro reaction. The amount of alcohol, acetaldehyde and acetic acid present at any given stage of the process depends upon the oxygen supply, the pH of the medium, the temperature and species of Acetobacter.

ii. Fermentation Conditions

At temperatures below 12° to 15° C. acetic acid bacteria grow slowly, and at temperatures of 42° to 45° C. produce cells that lose the power to function normally. From 15° to 34° C. Acetobacter appear to develop normally. The organism and procedure employed during fermentation determine the exact temperature to be used. Too low a temperature favours

a slow fermentation, but too high a temperature favours the loss through evaporation of alcohol, acetic acid and the volatile substances important in the production of flavour and aroma (27).

Since the conversion of ethyl alcohol to acetic acid is an oxidative reaction, large quantities of air must be present during fermentation. Some species of Acetobacter oxidize acetic acid to carbon dioxide and water; these are undesirable for fermentation. Some species in a limited amount of air produce acetic acid, but in a large amount of air produce carbon dioxide and water. Therefore, the amount of oxygen must be controlled in relation to the activities of the bacterium causing the oxidation of ethyl alcohol. In practice Shimwell (35) claimed that over-oxidation often must be controlled by avoiding over-aeration and by stopping the process while there is still a little alcohol present. If the first generator was built at approximately 1670, the fact that oxygen is necessary for acetic acid fermentation must have been known at that time. Pasteur showed that the fermentation must be arrested when fermentation is completed, otherwise the "mycoderms" transform the acetic acid into water and carbon dioxide (2). Tosic (45) found that with Acetobacter turbidans a lag period precedes the oxidation of acetate. Adenosine triphosphate slightly reduces the lag period; small concentrations of lactate, pyruvate, glycerol, succinate, malate, fumarate, a-ketoglutarate, glucose, formate and formaldehyde greatly reduce the lag period. Apparently the

effect of these substances may be due to the activation of one of the enzymes essential for oxidation of acetate.

iii. Pure Cultures

Although many investigators have recommended the use of pure cultures of acetic acid bacteria for the production of vinegar, such practice has not been popular. The construction of generators, storage tanks and other equipment in common use for the manufacture of vinegar does not favour sterilization of such installations. Shimwell (35) claimed that the use of a pure culture of species possessing the most desirable characteristics is advantageous. The organism should be able to tolerate a higher concentration of alcohol, convert ethyl alcohol to acetic acid rapidly, oxidize ethyl alcohol incompletely and produce good-flavoured vinegar. He stated that the maintenance of pure cultures and the exclusion of undesirable types is not, however, easily achieved with traditionally constructed acetifiers. In most instances, domination of the desirable type is more readily attained by the regulation of the conditions, particularly temperature and aeration. Several different species are usually present in commercial acetifiers; the proportions of these seem to settle down to a workable mixture which remains fairly constant for long periods. Shimwell claimed that the type of bacterium best suited for the "quick" vinegar process is one with a minimum tendency to over-oxidation, rapid utilization of high concentrations of alcohol and a minimum

of film production; Acetobacter schuetzenbachii and Acetobacter curvum possess these properties and were first isolated by Henneberg from the "quick" vinegar process. Shimwell (38) claimed that the nature of acetic acid bacteria, responsible for producing vinegar in industrial operations, has until now remained unknown. He isolated a pure culture of the "true working bacteria" in a vinegar acetifier and described the morphological, cultural and physiological characteristics of the pure culture. The pure culture was used successfully in making spirit-, malt-, and wine-vinegars. Acetification was rapid and the vinegar produced was in no way inferior in aroma and flavour to vinegar produced by the empirical mixed culture method. In using pure cultures in any industrial fermentation, the culture should be able to stand prolonged sub-cultivation in the laboratory without changing its characteristics. The strain described did not show signs of losing its acid tolerance properties. Possibly uniformity of product is the greatest advantage gained by the use of pure cultures.

4. Summary and Conclusions

1. Vinegar has been made biologically for centuries. Louis Pasteur's experiments confirmed the living nature of "mother of vinegar" as the active agent in acetification. Hansen first recognized that several species of vinegar bacteria are able to cause beer to turn sour.
2. Various names have been used in the past to designate organisms that are known today as Acetobacter. The genus has been allocated to several different families; the most suitable is Pseudomonadaceae. The unvarying characteristic of the genus is the production of acetic acid from ethyl alcohol in acid media.
3. Materials available in the brewery, notably malt wort, beer and yeast, provide media of optimum composition for the cultivation of Acetobacter. Species of the genus favour acid media. In general, little work has been done on chemical factors affecting growth of a variety of Acetobacter organisms.
4. Many species of Acetobacter have been described. Isolated species have been lost, some have been named, and may be strains of the same species of organisms isolated and named differently later. Single organisms show variations in behaviour. The difficulty has been to find unvarying characteristics from which to plan a classification of species.

5. The production of acetic acid from ethyl alcohol by Acetobacter is believed to be entirely an oxidative reaction; that is, ethyl alcohol is oxidized to acetaldehyde, and acetaldehyde is oxidized to acetic acid.
6. Two methods are used in vinegar production. In the "slow" process alcohol-containing media is allowed to be acidified by Acetobacter species; the liquid must be kept still. In the "quick" process alcohol-containing media is allowed to be acidified by Acetobacter species by continuously passing the liquid through a generator containing a percolating medium in order to increase the surface exposed to air.
7. Vinegar production is dependent on the availability of nutrients required by the organisms. Species of Acetobacter vary in their ethyl alcohol tolerance. Usually 10 to 13 per cent ethyl alcohol is converted to acetic acid. The effect of the pH of the initial medium on acid production has not been studied.
8. The organisms develop normally at temperatures of 15° to 34°C. The exact temperature used during fermentation depends on the organism and the procedure. Oxygen must be present during the fermentation. The amount of oxygen depends on the organism used.
9. The use of a pure culture of Acetobacter to effect fermentation is advantageous. The pure culture could provide uniformity of the product, if the strain used does not show marked variation in behaviour.

D. EXPLORATORY METHODS AND RESULTS

1. A Study of the Basic Characteristics of Acetobacter

a. Propagation Media

During preliminary experiments two species of *Acetobacter* were used, *Acetobacter suboxydans* and an unknown species which was later identified as *Acetobacter aceti* 2.[≁] Two different media were tested for growth of the organisms to determine which would be better for subculture. They had the following ingredients:

Yeast extract glucose agar

Difco's Bacto-Yeast Extract	10 gm.
Agar	15 gm.
Glucose	10 gm.
Tap water	1000 ml.

Wort agar

Difco's Wort Agar	50 gm.
Tap water	1000 ml.

Each medium was made in the usual way, dissolving the agar in the steamer, distributing it in 10 ml. quantities in test tubes, autoclaving, and allowing it to harden as agar slopes. The slopes were inoculated with the respective organisms, and the resulting growths were compared (Table II). Yeast extract glucose agar was the better medium.

≁ - Macdonald College Collection.

TABLE II

GROWTH OF ACETOBACTER SPECIES ON YEAST EXTRACT GLUCOSE AGAR AND WORT AGAR

Organism	Yeast extract glucose agar	Wort agar
Acetobacter suboxydans	✓[a] (48 hrs.)	o[b]
Acetobacter aceti 2	✓ (48 hrs.)	✓ (72 hrs.)

a - ✓ - growth.
b - o - no growth.

When standard cultures of Acetobacter were obtained sub-cultures were made every 14 days on yeast extract glucose agar slopes. One-tenth per cent of calcium carbonate was added to medium, in order to neutralize any acid formed. In later work the medium will be referred to as yeast extract glucose agar containing 0.1 per cent calcium carbonate.

During experiments the Acetobacter species grew well on the yeast extract glucose agar containing 0.1 per cent calcium carbonate, but later growth became very scant. Acetobacter melanogenum 2 would not grow, and Acetobacter melanogenum 1 grew very scantily. Therefore Difco's Micro Assay Culture Agar, 47 gm. dissolved in 1000 ml. tap water, was used. The specific results are not recorded. In general some organisms grew more profusely and in a shorter interval of time, while others grew very scantily.

Comparative tests were made by culturing organisms on the two following media. The growth results are recorded in Table III.

TABLE III

GROWTH OF ACETOBACTER SPECIES ON YEAST EXTRACT AGAR CONTAINING 2 PER CENT CALCIUM CARBONATE AND ON DIFCO'S "STOCK CULTURE AGAR"

Organisms	Yeast extract glucose agar containing 2% calcium carbonate	Difco's "Stock Culture Agar"
A. suboxydans	////[c]	/
A. acetosum	////	//[d]
A. ascendens	////	/
A. pasteurianum	////	//
A. turbidans	////	///
A. kützingianum	////	/
A. aceti 1	////	o
A. aceti 2	////	////
A. rancens	////	o
A. gluconicum	///	o
A. melanogenum 1	///	o
A. oxydans 1	////	///
A. oxydans 2	////	////
A. xylinum	/	o
A. peroxydans	/	/

c - //// - very good growth.
d - // - good growth.

Yeast extract glucose agar containing 2 per cent calcium carbonate

Difco's Bacto-Yeast Extract	10 gm.
Agar	15 gm.
Glucose	10 gm.
Calcium carbonate	20 gm.
Tap water	1000 ml.

Difco's "Stock Culture Agar"

Difco's Stock Culture Agar	25 gm.
Tap water	500 ml.

The results indicated that the yeast extract glucose medium containing 2 per cent calcium carbonate was the better. This medium, therefore, was used for subsequent cultures. Sub-cultures were made every two weeks. The organisms were allowed to grow for three days at room temperature, and then stored in a refrigerator until required.

b. Identification of Genus

Acetobacter species may be identified by their morphology, reaction to Gram's staining method, and ability to oxidize ethyl alcohol to acetic acid in the presence of an appropriate basal medium. Marshall and Walkley (23) described a method for isolating and identifying the organisms on apples. They determined the number of yeasts, acetic acid, and lactic acid organisms present. In tests to identify the bacteria they only mentioned that acetic acid and lactic acid organisms were differentiated by Gram's staining methods and the catalase reaction. Marshall and Walkley's

tests were used to identify Acetobacter in plant juices; if the appropriate results were obtained, the ability of each organism to oxidize ethyl alcohol was tested.

The efficiency of the tests in identifying organisms as members of the genus Acetobacter was determined with two species known to be organisms of the genus, Acetobacter suboxydans and an unknown Acetobacter species later identified as Acetobacter aceti 2/. After obtaining the required results, organisms isolated from maple syrup vinegar were tested similarly to determine whether Acetobacter species were present. The organisms were grown on yeast extract glucose agar slopes and cells of the resulting growths were stained by Gram's method. The cells of both species were Gram-variable rods, but those of the second were much thinner in appearance. To test for catalase 3 per cent hydrogen peroxide was added to a forty-eight-hour slope of each culture. The evolution of gas with each culture indicated a positive catalase test. To test for acetic acid production 100 ml. of malt extract medium in a 250 ml. Erlenmeyer flask was inoculated with one loopful of a forty-eight-hour culture. The malt extract broth was prepared by placing 15 gm. of Difco's Malt Extract in 970 ml. tap water, dissolving in a steamer and autoclaving for 15 minutes at 15 lb. pressure. Thirty milliliters of 95 per cent ethyl alcohol was added when the medium was cool, and approximately 100 ml. quantities were added to 250 ml. sterile Erlenmeyer flasks. Acetic acid production after inoculation with test organisms was determined by

/ - Macdonald College Collection.

titration with 0.1 normal sodium hydroxide using phenolphthalein as indicator, and by odour. Both Acetobacter suboxydans and the unknown species produced the characteristic vinegar odour in the medium. A loopful of the vinegar made from maple syrup was plated with yeast extract glucose agar. Five organisms were isolated. All were Gram-negative rods and gave a positive catalase test. Only one of the organisms, however, oxidized ethyl alcohol to acetic acid in the malt extract medium. The reaction of Acetobacter to Gram's staining method and the oxidation of ethyl alcohol to acetic acid were considered the most important tests.

c. Isolation of Acetobacter from Apples

An attempt was made to isolate Acetobacter species from apples. The method prescribed by Marshall and Walkley (23) was modified and used. Each apple, obtained aseptically from a tree, was quartered with a sterile knife, and with 400 ml. of sterile water put into a sterile Waring blender and comminuted for two minutes at few-second intervals. One milliliter samples of various dilutions of the mixture were placed in sterile Petri dishes. Ten milliliters of medium, made according to the following directions, was poured into each plate. Commercial unsweetened apple juice was diluted to reduce the sugar concentration to 2 per cent. To 500 ml. of the diluted juice 1 gm. of ammonium phosphate and 10 gm. of powdered agar were added, and the mixture was heated in a steam sterilizer until dissolved. It was then placed in

10 ml. quantities into tubes and sterilized in the autoclave. After plating samples from 2 apples, 0.1 per cent yeast extract was added to enhance the growth of yeasts and bacteria. Inoculated plates were incubated five days at 25°C. Colonies suspected to be Acetobacter were isolated by spreading them on yeast extract glucose agar plates, and then subculturing pure colonies on yeast extract glucose agar slopes.

Positive identity of Acetobacter was established by inoculating 100 ml. quantities of malt extract alcohol broth (page 44) in 250 ml. Erlenmeyer flasks with Gram-negative organisms, suspected to be Acetobacter.

Twenty-one apples were tested by the technique and acetic acid bacteria were not isolated. Waring blender mash from four apples was added in 5 ml. quantities to 100 ml. of malt extract alcohol broth in flasks (49). A vinegar odour developed in one flask. A loopful of the broth in the latter flask was streaked onto a yeast extract glucose agar plate, and after subculturing the different types of colonies of Gram-negative rod-shaped bacteria in flasks containing malt extract alcohol broth an Acetobacter was isolated and identified.

Although an efficient method of isolating acetic acid bacteria had been found, the apple season had ended. Acetobacter species, obtained from the American Type Culture Collection in Washington, D.C. were used for research on the oxidative powers of Acetobacter species in the presence of ethyl alcohol.

d. Identification of Species

In the preceding pages a method for identification of Acetobacter was described. Three organisms, an unknown Acetobacter species that had been given by the Department of Bacteriology, a species isolated from the maple syrup vinegar, and one isolated from the apples studied, had to be classified. As identification has not been studied intensively and much controversy exists among workers on supposedly established factors important in classification, the preliminary classification in Bergey's Manual, 6th edition (5), was used.

Vaughn (49) suggested a method for studying the nature of oxidation of ethyl alcohol by Acetobacter. He claimed that compounds such as ethyl alcohol and acetic acid, which may be completely oxidized, could be tested in yeast water-agar containing 2 per cent calcium carbonate. Acid production is indicated by clearing of the carbonate and carbon dioxide production by the reprecipitation of the calcium. The method described by Vaughn was used to study the oxidation of ethyl alcohol and acetic acid by Acetobacter, but the medium was slightly changed. Ten grams of Difco Bacto Yeast Extract, 20 gm. of calcium carbonate, and 15 gm. of agar were diluted with 1000 ml. of tap water and dissolved in a steamer, dispensed in 10 ml. quantities into test tubes and autoclaved. Before pouring into sterile Petri

dishes 2 per cent ethyl alcohol was added to each test tube but not to the control tubes. One loopful of a forty-eight-hour culture of each organism was suspended in 10 ml. of sterile water, and one loopful of the suspension was placed in three locations on a plate containing the medium.

Henneberg described a medium containing ammonium salts as a sole source of nitrogen (49). The medium was used in the present investigation to determine the ability of the organisms to utilize ammonium salts as sole source of nitrogen, and was prepared as described. Three grams of ammonium sulphate, 3 gm. of primary potassium phosphate, 2 gm. of magnesium sulphate, and 20 gm. of glucose were dissolved in 1000 ml. of tap water, dispensed in 9.8 ml. quantities in test tubes, and autoclaved at 15 lb. pressure for fifteen minutes. Before use, 95 per cent ethyl alcohol was added to give a final concentration of 2 per cent in the medium. Forty-eight-hour cultures grown on yeast extract glucose agar slopes containing 0.1 per cent calcium carbonate were suspended in 10 ml. of sterile distilled water. The suspension of each organism was centrifuged three times; the supernatants were decanted and the organism resuspended in 10 ml. of liquid, the first liquid being a 0.9 per cent sodium chloride solution, and the others distilled water. One loopful of the final suspension was added to the described medium.

Slight changes in Vaughn's methods (49) were made for the study of pigment and membrane formations. Pigment in

glucose medium was determined on the yeast extract glucose agar slopes containing 0.1 per cent calcium carbonate. Membrane formation was studied in malt extract alcohol broth (page 44).

The three Acetobacter species were tested with the appropriate described tests in accordance with Bergey's classification. The results are recorded in Table IV. The unknown species from the Department was Acetobacter aceti, the Acetobacter species isolated from maple syrup vinegar was Acetobacter xylinum, and the one isolated from the apples was Acetobacter melanogenum.

2. Acetobacter Species under Investigation

In the comparative work that will be described in future the following organisms were used:

ATCC	8303	Acetobacter aceti 1
"	6438	Acetobacter acetosum
"	9323	Acetobacter ascendens
"	9324	Acetobacter gluconicum
"	6439	Acetobacter kützingianum
"	9937	Acetobacter melanogenum 1
"	9433	Acetobacter oxydans
"	6033	Acetobacter pasteurianum
"	838	Acetobacter peroxydans
"	7839	Acetobacter rancens
"	9325	Acetobacter turbidans

ATCC - The American Type Culture Collection, Washington, D.C.

TABLE IV

IDENTIFICATION OF UNKNOWN *ACETOBACTER* SPECIES

Organism	Oxidation of ethyl alcohol	Growth in ammonium salts	Pigment formation in glucose medium	Membrane formation in ethyl alcohol-containing medium	Species
Acetobacter from Dept. of Bact. (89)	Clearing and reprecipitation	+			*Acetobacter aceti*
Acetobacter from maple syrup (M of V)	Clearing and reprecipitation	o		Like cellophane, thick and transparent	*Acetobacter xylinum*
Acetobacter from apple (XXI)	Clearing		dark brown		*Acetobacter melanogenum*

MCC ##	109	Acetobacter suboxydans
"	89	Acetobacter aceti 2
###	XXI	Acetobacter melanogenum 2
###	MV	Acetobacter xylinum

3. A Study of Three Methods of Acetic Acid Production from Ethyl Alcohol by Acetobacter

a. Preliminary Methods

i. Preparation of Inocula

Inocula were prepared in a definite way to have the results of experiments relative to one another. Unless indicated otherwise the following procedure was used. Each organism was grown for forty-eight hours at room temperature, or seventy-two hours when necessary, on slopes of stock culture medium, which in the final experiments was yeast extract glucose agar containing 2 per cent calcium carbonate. Each forty-eight-hour slope was washed with 8 ml. of sterile water, and the suspension obtained was poured into a sterile centrifuge tube. The suspension of each organism was

- Macdonald College Collection.
- See Table IV, page 50.

centrifuged three times; the supernatants were decanted and the organism resuspended in 8 ml. of liquid; the first liquid being a 0.9 per cent sodium chloride solution, and the others water. The final suspension was used for inocula; and for testing the viability of the organism on yeast extract glucose agar slopes.

During some of the preliminary experiments only water washings were used. Due to fragility of the organisms and various investigators' recommendation, normal saline was finally used. Tap water suspensions were made. When synthetic media were to be inoculated, however, distilled water suspensions were made. One loopful of the final suspension was cultured on a yeast extract glucose agar slope to test for the viability of the organism.

ii. Determination of the Amount of Acetic Acid

When broth culture media were used for acetic acid production from ethyl alcohol, 10 ml. of inoculated test culture medium was titrated at various intervals with 0.1 normal sodium hydroxide with phenolphthalein as the indicator. Calculation of the amount of acetic acid present was computed according to the method of "Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists" (18); 1 ml. of 0.1 normal sodium hydroxide was claimed to equal 0.006 gm. of acetic acid. To calculate the amount of acetic acid present in 100 ml. of medium,the amount of

0.1 normal sodium hydroxide required to neutralize 10 ml. of the control medium was subtracted from the amount of 0.1 normal sodium hydroxide required to neutralize the test medium and the resultant figure was multiplied by 0.06.

b. Method I

Adaptation of the Perfusion Apparatus Designed by Audus to Simulate Conditions of the "Quick" Process

i. Procedure

An apparatus that had been designed by Audus for studies of nitrification was used in an attempt to construct conditions similar to the "generator" process. A diagram of the apparatus is on page 54. The apparatus was assembled and sterilized by methods that will be described on the following pages. All parts were sealed with collodion. Cellulose sponge or glass beads were added to the glass tube. Sponge, cut in 10 cm. lengths and fitting well in the glass tube, was used to advantage previously by the Department of Bacteriology, and was therefore used in the experiments. A small glass rod prevented the sponge from obstructing the hole at the bottom of the glass tube. When glass beads were used, a small quantity of glass wool over the glass rod aided the same purpose. Sterile media and the organism to be tested were added by the side arm. The most satisfactory amount of medium was 150 ml. The small glass tubing inside the reservoir but connecting the apparatus

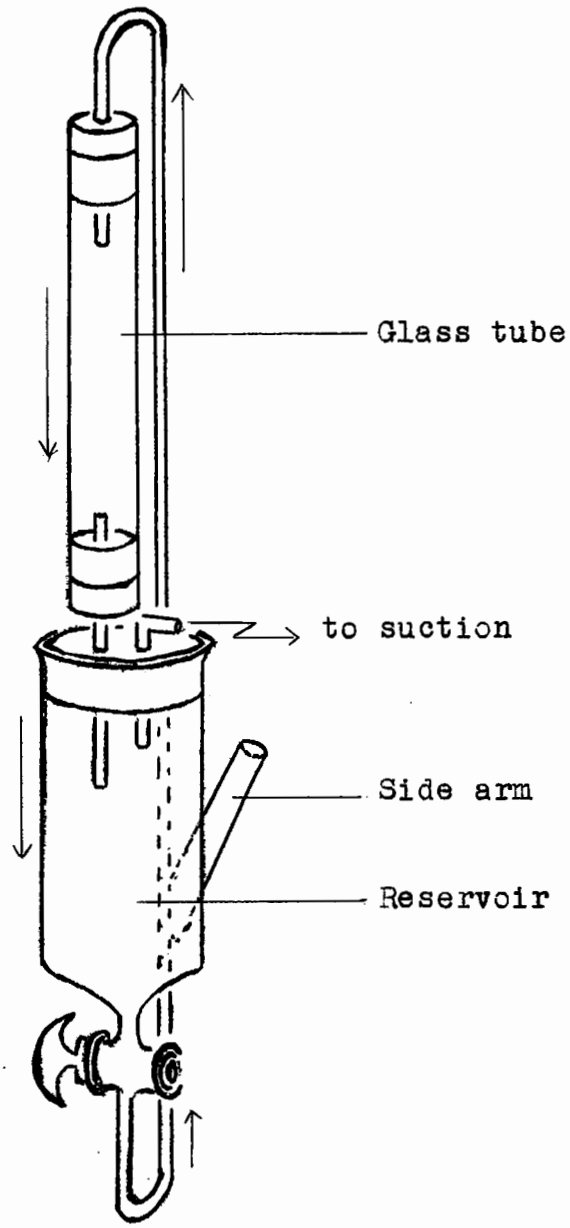


FIGURE 1 - PERFUSION APPARATUS

to the suction was as short as possible, and the small glass tubing connecting the glass tube with the reservoir was as long as possible without touching the level of the liquid in the reservoir in order to keep media from escaping through the suction tube. Rubber tubings connected eight similar apparatuses in series to a long glass tubing, which was connected to a suction. Any apparatus could be disconnected from the series by using a stopcock on the rubber tubing of the appropriate apparatus. When an apparatus was in operation, the suction pulled the inoculated medium through the apparatus in the direction indicated by the arrows on the figure. The organisms adhered to the cellulose sponge or glass beads and thus converted the ethyl alcohol in the percolating medium to acetic acid.

ii. Acid Production with *Acetobacter Suboxydans* in Various Nutrient Media

Fermentation apparatuses, including 10 cm. sponges, were washed in Calgonite, a detergent. Glassware and sponges were rinsed in tap water and in distilled water. Sponges were allowed to dry overnight in the oven. Glass tubes containing the sponges and cotton plugs at each end, stopcocks wrapped in paper, and cotton plugs in clean test tubes to be used later for insertion in the side arms, were autoclaved. All other parts of the apparatuses were sterilized by rinsing three times with 95 per cent ethyl alcohol.

Henneberg's basal glucose disodium phosphate medium (19) with minor changes was used.

Water	930.0 ml.
Magnesium sulphate	2.0 gm.
Dibasic potassium phosphate	3.0 gm.
Sodium chloride	2.0 gm.

The effect of 1.0 gm. of Difco's Bacto Yeast Extract, 1.0 gm. of casein hydrolysate and indicated synthetic media, respectively, on acetic acid production by Acetobacter suboxydans was determined in the described salts solution. The media were adjusted to pH 6, with bromthymol purple as indicator, and sterilized in the autoclave for fifteen minutes at 15 lb. pressure. Seventy ml. of 95 per cent ethyl alcohol were added before each medium was transferred to each apparatus.

The media, in 150 ml. quantities, were added to each apparatus by means of a sterile 50 ml. pipette. The test culture, Acetobacter suboxydans, was grown on yeast extract glucose agar slopes containing 0.1 per cent calcium carbonate and prepared as described on page 51. One milliliter of the final suspension of the organism was added to each apparatus containing a medium, but not to the controls. Control media were used to estimate possible contamination of the apparatuses after manipulation. Acetic acid production was determined as described on page 52. Sterile media were added to apparatuses when the level of the liquids diminished. Additions are indicated on appropriate tables of results.

Experiments 1 and 2

Tables V and VI show the amounts of acetic acid produced from 7 per cent ethyl alcohol in media containing yeast extract and casein hydrolysate, respectively, as sole source of nitrogen at various intervals of time. The results indicate that Acetobacter suboxydans produced more acetic acid in the yeast extract medium than in the casein hydrolysate medium. Taking the titration figures in the control apparatuses as indication of contamination, the conclusion is that the apparatuses when set up were sterile. The changing of titration figures indicated that contamination occurred after the third day. Contamination may have been due to leaks in the apparatuses or manipulation of apparatuses in adding or taking away media.

Experiment 3

The effect was determined of a synthetic medium containing indicated amino acids and growth factors in place of yeast extract or casein hydrolysate on acetic acid production. Amino acids were grouped according to the similarity of their formulae (53). For the experiment each component of each of the seven groups was used in 0.2 gm. amount in 1000 ml. of medium. The components were alanine, glycine, norleucine, serine, threonine, cysteine, methionine, taurine, isoleucine, leucine, valine, aspartic acid, glutamic acid, arginine, asparagin, lysine, ornithine, histidine, hydroxyproline, proline, phenolalanine,

TABLE V

ACETIC ACID PRODUCTION FROM 7 PER CENT ETHYL ALCOHOL BY ACETOBACTER SUBOXYDANS WITH YEAST EXTRACT AS SOLE SOURCE OF NITROGEN

Time, days	Apparatus 1 Test titration, ml	[e]HAc/100 ml. medium, gm	Apparatus 2 Control titration, ml	Apparatus 3 Test titration, ml	HAc/100 ml. medium, gm	Apparatus 4 Control titration, ml	Sterile medium added to apparatuses
1	1.6	0.000	1.6	1.6	0.000	1.6	
2	1.9	0.018	1.6	2.0	0.024	1.6	
3	3.0	0.084	1.6	3.6	0.120	1.6	
6	6.1	0.270	2.0	7.2	0.336	1.7	
8	7.4	0.348	1.5	8.0	0.384	1.55	10 ml. to app.[f] 1,2,3,4
9	8.2	0.396		9.2	0.456	1.4	10 ml. to app. 1 and 3
10	8.7	0.426		9.61	0.4806	1.45	10 ml. to app. 1 and 4
11	9.6	0.480		11.8	0.612		

e - HAc - Acetic acid.
f - App.- Apparatus.

TABLE VI

ACETIC ACID PRODUCTION FROM 7 PER CENT ETHYL ALCOHOL BY ACETOBACTER SUBOXYDANS WITH CASEIN HYDROLYSATE AS SOLE SOURCE OF NITROGEN

Time, days	Apparatus 1 Control titration, ml	Apparatus 2 Test titration, ml	HAc/100 ml. medium, gm	Apparatus 3 Test titration, ml	HAc/100 ml. medium, gm	Apparatus 4 Test titration, ml	HAc/100 ml. medium, gm	Sterile medium added to apparatuses
1	1.55	1.55	0.000	1.55	0.000	1.55		10 ml. to app. 1,2,3,4
2	1.55	1.55	0.000	1.70	0.009	1.65	0.006	10 ml. to app. 1,2,3,4
3	1.55	1.60	0.003	1.80	0.015	1.70	0.009	-
4	1.70	1.75	0.012	1.85	0.018	1.70	0.009	10 ml. to app. 1,2,3,4
6	2.00	1.80	0.015	2.00	0.027	1.80	0.015	30 ml. to app. 1,2,3,4
10	2.05	1.65	0.006	3.20	0.099	5.60	0.243	-

tryptophane and tyrosine. Solutions were made in distilled water of the following growth factors:

p-Aminobenzoic acid - 1 ml. solution contains 10.0 µg.

Biotin - 1 ml. solution contains 0.1 µg.

Inositol - 1 ml. solution contains 0.05 gm.

Nicotinic acid - 1 ml. solution contains 100.0 µg.

Pantothenic acid - 1 ml. solution contains 100.0 µg.

Pyridoxine - 1 ml. solution contains 200.0 µg.

Riboflavin - 1 ml. solution contains 200.0 µg.

Thiamine - 1 ml. solution contains 100.0 µg.

One milliliter of each was used in 1000 ml. of medium. The complete medium was dissolved as much as possible by heating in the steamer and over the flame before it was autoclaved. The remaining procedure was that described on pages 55 and 56.

Table VII shows the amount of acetic acid produced at various intervals of time. Acetobacter suboxydans did not produce acid. Control apparatuses were sterile until the third day.

Experiment 4

The effect was determined of a medium, prepared as described in Experiment 3 but containing the indicated vitamins and purines and pyrimidine bases, on acetic acid production.

p-Aminobenzoic acid - 1 ml. solution contains 10.0 µg.

Biotin - 1 ml. solution contains 0.1 µg.

Vitamin B-12 - 1 ml. solution contains 0.015 µg.

Folic acid - 1 ml. solution contains 2.0 µg.

Inositol - 1 ml. solution contains 0.05 gm.

Nicotinic acid - 1 ml. solution contains 100.0 µg.

Pantothenic acid - 1 ml. solution contains 100.0 µg.

Pyridoxine - 1 ml. solution contains 200.0 µg.

Riboflavin - 1 ml. solution contains 200.0 µg.

Thiamine - 1 ml. solution contains 100.0 µg.

Adenine sulphate - 1 ml. solution contains 20.0 mg.

Guanine - 1 ml. solution contains 20.0 mg.

Uracil - 1 ml. solution contains 20.0 mg.

Xanthine - 1 ml. solution contains 20.0 mg.

Table VIII shows the amount of acetic acid produced at various intervals of time. Acetobacter suboxydans did not produce acid. Control apparatuses were sterile throughout the test.

iii. Interpretation of Experimental Results

The results may have been accurate, as some amino acids and growth factors could possibly have an inhibitory effect on the oxidation of ethyl alcohol. Definite evidence has been given that certain vitamins of the B-complex group inhibit the growth of Acetobacter xylinum (20). Also, certain essential substances may not have been used in making the media. It was noted, however, that when any type of medium was perfused

TABLE VII

ACETIC ACID PRODUCTION FROM 7 PER CENT ETHYL ALCOHOL BY ACETOBACTER SUBOXYDANS WITH THE AMINO ACIDS AS SOLE SOURCE OF NITROGEN IN THE PRESENCE OF GROWTH FACTORS (See Experiment 3)

Time, days	Apparatus 1 Test titration, ml	Apparatus 1 HAc/100 ml. medium, gm	Apparatus 2 Control titration, ml	Apparatus 3 Test titration, ml	Apparatus 3 HAc/100 ml. medium, gm	Apparatus 4 Control titration, ml	Sterile medium added to apparatuses
1	1.85	0.000	1.85	1.85	0.000	1.85	10 ml. to app. 1,2,3,4
2	1.85	0.000	1.85	1.85	0.000	1.85	10 ml. to app. 1,2,3,4
3	1.85	0.000	1.85	1.85	0.000	1.85	-
4	1.85	0.000	1.90	1.90	0.003	1.90	10 ml. to app. 1,2,3,4
5	2.20	0.021	1.85	2.00	0.009	1.90	-
6	2.35	0.030	1.85	2.00	0.009	1.85	
7	1.00	-	1.90	2.00	0.009	1.85	
9	1.30	-	1.00	1.75	-	2.00	

TABLE VIII

ACETIC ACID PRODUCTION FROM 7 PER CENT ETHYL ALCOHOL BY ACETOBACTER SUBOXYDANS WITH THE AMINO ACIDS AS SOLE SOURCE OF NITROGEN IN THE PRESENCE OF GROWTH FACTORS (See Experiment 4)

Time, days	Apparatus 1 Control titration, ml	Apparatus 2 Test titration, ml	Apparatus 3 Test titration, ml	Apparatus 4 Control titration, ml	Each test HAc/100 ml. medium, gm	Sterile medium added to apparatuses
1	1.4	1.35	1.35	1.4	0.00	10 ml. to app. 1,2,3,4
2	1.4	1.40	1.40	1.4	0.00	10 ml. to app. 1,2,3,4
3	1.4	1.40	1.40	1.4	0.00	10 ml. to app. 1,2,3,4
4	1.4	1.40	1.40	1.4	0.00	

through the apparatuses and Orvis, a synthetic detergent, had been used to wash the apparatuses and sponges, a slight cloudiness appeared in the liquid dropping from the sponges in the glass tube to the reservoir. The new soap may have added a cloudy material to the sponges and the medium may have extracted it. The material may have prevented Acetobacter suboxydans from oxidizing ethyl alcohol.

Numerous methods were used to clean the sponges in order to eliminate the cloudy material. Only eight apparatuses could be set up at once. Time was required to find what effect the method had on acid production by Acetobacter suboxydans. During all experiments Acetobacter suboxydans never produced acid from ethyl alcohol even in the yeast extract medium. Finally specimens of nutrient in the sponges were taken and plated in order to determine whether the organisms were still alive. Since they were alive, they may have produced mutations incapable of acetic acid production. Using Method II, as will be described on page 70, the same conclusion was evident within 48 hours after beginning the test.

iv. Experimental Work to Improve Method I

Experiment 1

Apparatuses were sterilized in an oven in an attempt to improve the method of sterilization. Each part of two apparatuses was washed and assembled ready for use. The stopcocks were replaced by cotton plugs due to probable liquefaction of

the grease in the oven. The apparatuses were maintained in an oven for one hour at 160 - 170° C. The stopcocks were sterilized separately. Oven sterilization proved unsuccessful as the rubber stoppers and tubing melted.

Experiment 2

Apparatuses were autoclaved in an attempt to improve the method of sterilization. Two apparatuses and sponges were washed with detergent and rinsed in tap water. Each apparatus, with the exception of the stopcock, was completely assembled. The stopcock receptacle was closed with a cotton plug, and a piece of paper was placed at the upper end of the receptacle and tied with a string. The procedure allowed for the later sterile insertion of the stopcock. Cotton plugs were placed in the side arm and in the suction tubing. The stopcocks were wrapped in paper. Each apparatus and stopcock was autoclaved for fifteen minutes under 15 lb. pressure. Each stopcock was inserted aseptically after removing the cotton and paper from each receptacle and placing grease on the inside upper end of the receptacle and on the lower end of the stopcock. All joints were sealed with collodion.

Acetobacter oxydans was cultured for forty-eight hours on a yeast extract glucose agar slope containing 0.1 per cent calcium carbonate, then washed as described on page 51. A one ml. quantity of suspension was added to each medium before the latter was added to the apparatuses. One loopful

of the suspension was streaked on a yeast extract glucose agar slope containing 0.1 per cent calcium carbonate to test the viability of the organism.

The media used in each apparatus were prepared separately in 500 ml. flasks. They had the following content:

	Flask 1	Flask 2
Yeast extract	1.5 gm.	1.5 gm.
Tap water	150.0 ml.	135.0 ml.
Ethyl alcohol	0.0 ml.	15.0 ml.

The flasks were covered with rubber stoppers containing a plugged hollow glass tubing in order that after sterilization media could be added directly to the apparatuses from the flasks. The solution of yeast extract and water in each flask was adjusted to a pH 6 before sterilization. After sterilization 15 ml. of ethyl alcohol was added to Flask 2, and 1 ml. of bacterial suspension to each of Flasks 1 and 2. The control medium did not contain ethyl alcohol. It was inoculated, however, with the test bacterium. Titration readings were assumed to indicate the amount of acids other than acetic acid formed by the bacterium.

After each 10 ml. quantity was taken from each apparatus for titration, 10 ml. of identical sterile medium was replaced. Because the lowering of the level of the liquid in the reservoir was believed to be due to evaporation, the level was maintained with sterile tap water. The amount of acetic acid present was calculated by the method described on page 52.

The results are given in Table IX. Acetobacter oxydans produced 4.06 gm. of acetic acid in seventy-two hours. The amount of acid present in the fermenting medium then decreased. Although the medium became clear in the test apparatus and the amount of acid decreased after 144 hours, Acetobacter organisms, isolated from the sponges, were grown on a yeast extract glucose agar plate containing 0.1 per cent calcium carbonate. Therefore, the decrease in the amount of acetic acid present in the fermenting broth was due possibly to the evaporation of ethyl alcohol and acetic acid. Although media in the apparatuses could have become contaminated due to the manipulation of the apparatuses during experiments, the only other source of contamination was the stopcock grease, which had not been sterilized.

Experiment 3

Another attempt was made to maintain sterile conditions in the apparatuses during experiments. The apparatuses were set up as described in Experiment 2. Swabs dipped in stopcock grease were placed, however, in test tubes and autoclaved. The stopcocks were lubricated with the sterile grease. With the latter procedure the apparatuses were completely sterile. Similar media were prepared and added to the apparatuses in the same way. Acetobacter aceti 1 was used for the test.

TABLE IX

ACETIC ACID PRODUCTION FROM 10 PER CENT ETHYL ALCOHOL BY ACETOBACTER OXYDANS WITH YEAST EXTRACT AS SOLE SOURCE OF NITROGEN

Time, hours	Apparatus 1 Control titration, ml	Apparatus 2 Test titration, ml	HAc/100 ml. medium, gm	Sterile medium added to apparatuses	Sterile water added to apparatuses
12	0.60	0.700	0.0060	10 ml. to each	
24	0.60	0.825	0.0135	10 ml. to each	
36	0.35	1.100	0.0450	10 ml. to each	10 ml. to each
48	0.15	10.800	0.6390	10 ml. to each	
60	0.00	46.150	2.7690	10 ml. to each	10 ml. to each
72	0.00g ie.-0.6	67.000	4.0560	10 ml. to each	
84	0.00g ie.-0.3	64.150	3.8670	10 ml. to each	
96	0.00g ie.-0.3	69.950	4.2150	10 ml. to each	
120	0.00g ie.-0.15	68.600	4.1250	10 ml. to each	
144	0.00g ie.-0.2	62.350	3.7530	10 ml. to each	
192	0.00	37.550	2.2530		
312		2.870	0.1722		

g - less than.

The results are given in Table X. Acetobacter aceti 1 produced acetic acid at a slower rate than Acetobacter oxydans, and did not produce a maximum amount of acid as large as that produced by Acetobacter oxydans (Table IX, page 66). After reaching a maximum value, the amount of acetic acid present in the fermenting broth decreased.

Experiment 4

Three complete apparatuses were assembled as described in Experiment 2. Glass beads, however, were used in place of cellulose sponges. Glass beads were arranged in 7.5, 10.0, and 13.0 cm. columns respectively. Yeast extract medium containing 10 per cent ethyl alcohol was used in the reservoirs and Acetobacter aceti 1 as the test bacterium.

Titration results are given in Table XI. The percolation of the medium through the apparatuses was very slow due to the compactness of the beads. The test, therefore, was discontinued.

v. Discussion

Through the experiments a method of oxidation of ethyl alcohol comparable with the "quick" vinegar process was obtained. The advantage of the procedure in the laboratory is indicated by the size of the apparatus and the fact that

TABLE X

ACETIC ACID PRODUCTION FROM 10 PER CENT ETHYL ALCOHOL BY ACETOBACTER ACETI 1 WITH YEAST EXTRACT AS SOLE SOURCE OF NITROGEN

Time, hours	Apparatus 1 Control titration, ml	Apparatus 2 Test titration, ml	HAc/100 ml. medium, gm	Sterile medium added to apparatuses	Sterile tap water added to apparatuses
18	0.70	0.80	0.006	10 ml. to each	
30	0.35	0.70	0.021	10 ml. to each	
42	0.15	0.80	0.039	10 ml. to each	
54	0.20	1.45	0.075	10 ml. to each	10 ml. to each
66	0.20	9.55	0.5718	10 ml. to each	10 ml. to each
78	0.20	21.15	1.257	10 ml. to each	10 ml. to each
90	0.20	29.25	1.743	10 ml. to each	
102	0.20	33.40	1.992	10 ml. to each	10 ml. to each
120	0.00[g]	42.80	2.568[h]	10 ml. to each	
146	0.00[g] -0.15	43.30	2.607	10 ml. to each	30 ml. to app. 1
170	0.00[g] -0.30	48.90	2.952		
218					30 ml. to each
266		24.30	1.458		

h - greater than.

TABLE XI

ACID PRODUCTION FROM 10 PER CENT ETHYL ALCOHOL BY *ACETOBACTER ACETI* 1 WITH YEAST EXTRACT AS SOLE SOURCE OF NITROGEN AND GLASS BEADS TO INCREASE AERATION SURFACE

Time, hours	Apparatus 1 7.5 cm. glass beads, test titration, ml	Apparatus 2 10.0 cm. glass beads, test titration, ml	Apparatus 3 13.0 cm. glass beads, test titration, ml
24	0.55	0.60	0.55
48	0.60	0.65	0.70
120	0.70	0.70	0.70

specific known organisms may be tested in a medium of known composition using cellulose sponge as a means of increasing surface area. The disadvantages for the present study are twofold. In studying the rate of oxidation and the amount of product formed, possibly much of both the ethyl alcohol and the acetic acid evaporate during the process. Also, because taps can only be in convenient places, the temperature, humidity and other physical factors cannot be controlled. Since these latter factors are of utmost importance in studying the rate of oxidation and the amount of product formed, the use of another method was necessary.

c. Method II

Calcium Carbonate Clearing Method

With Method I, Acetobacter species oxidized a relatively high percentage of ethyl alcohol. Much time was required, however, to assemble the apparatuses. Even with the greatest of care the possibility of contamination was great. When the procedure was thought to be unsuccessful during the experiments with Acetobacter suboxydans, the apparatuses were assembled numerous times in an attempt to solve the problem. As mentioned previously (page 47), Vaughn (49) claimed that when Acetobacter species oxidize ethyl alcohol to acetic acid in a medium containing yeast water, agar, alcohol and calcium carbonate, clear zones are produced in the calcium carbonate; when they oxidize acetic acid to carbon dioxide

and water, the calcium is reprecipitated. A modification of the procedure proved beneficial in studying the oxidation of ethyl alcohol by various unknown species in order to classify them. It was thought that the same method could be used to check some of the possible errors in the procedure used in Method I, and to study the rate of acetic acid production by Acetobacter species. A basic procedure was followed in order to investigate the possibilities of the "calcium carbonate clearing" method for these purposes.

i. Procedure

The basic medium for the work consisted of the following:

Difco's Bacto Yeast Extract	1.0 gm.
Agar	15.0 gm.
Calcium carbonate	20.0 gm.
Ethyl alcohol	30.0 ml.
Tap water	970.0 ml.

The yeast extract was dissolved in tap water and adjusted to a pH of 6. The calcium carbonate and agar were added to the solution, and the agar was then dissolved in the steamer. The medium was placed in approximately 10 ml. quantities in test tubes and autoclaved for fifteen minutes at 15 lb. pressure. The tubes of medium were cooled to 60° C., and 0.3 ml. of 95 per cent ethyl alcohol was added to each tube. The medium in each tube was thoroughly mixed and poured into sterile Petri dishes.

The organisms were prepared as described on page 51. One loopful of the final suspension was placed in each of three locations on the surface of the agar in each dish.

Readings of the diameter of each colony and clear zone they produced were noted.

ii. Interpretation of Experimental Results in Method I with Acetobacter Suboxydans

Three identical media, made and put into Petri plates as described, were prepared separately in glassware that had been washed in Aura, Orvis, and Calgonite, respectively, and rinsed with tap water. The detergents had been previously rinsed off the Petri plates in varying amounts in order to determine their possible inhibition of acetic acid production from ethyl alcohol. Three forty-eight-hour cultures of Acetobacter suboxydans were washed in water and sodium chloride in centrifuge tubes that had been washed with corresponding detergents. Duplicate test plates and a control containing no ethyl alcohol were inoculated with the organism prepared in centrifuge tubes washed with corresponding detergents.

The organism grew, but did not produce clear zones on any of the plates. The absence of clear zones, therefore, may have resulted from either an inadequate supply of yeast extract or a mutation of the organism. The latter seemed to be the more probable answer, and succeeding experiments

confirmed the conclusion. The organism had been subcultured for a long time on a medium consisting of glucose as a carbon source. It is possible, therefore, that this strain of Acetobacter suboxydans, may have lost the ability to oxidize ethyl alcohol.

iii. Standardization of Method

An attempt was made to standardize the plate method. Eleven plates were prepared as described on page 71. A normal solution of acetic acid was prepared. Dilutions were made as follows:

Test tube	1	2	3	4	5	6	7	8	9	10	11
Distilled water	10 cc.	9	8	7	6	5	4	3	2	1	0
Normal acetic acid	0 cc.	1	2	3	4	5	6	7	8	9	10

One loopful of the liquid in each tube was placed on each of three parts of a corresponding Petri plate. The plates were observed at frequent intervals for the degree of clearing of the calcium carbonate caused by the liquid.

Clear zones were not produced with acetic acid at any of the dilutions. Therefore, the results obtained in actual experiments were compared with those of a third method. The third method was believed to be quantitatively and qualitatively accurate.

iv. Growth of *Acetobacter* Organisms and Clearing of Calcium Carbonate on Plates Containing Various Nutrients

Experiment 1

The growth characteristics of *Acetobacter* species were determined on a medium containing 2 per cent ethyl alcohol and yeast extract as sole source of nitrogen. The procedure used was that described on page 71. The medium consisted of the following:

Difco's Yeast Extract	10 gm.
Agar	15 gm.
Calcium carbonate	20 gm.
Ethyl alcohol	20 ml.
Tap water	980 ml.

Ethyl alcohol was added to two-thirds of the tubes. Two test and one control plate were prepared for each organism. Fourteen organisms were grown for forty-eight hours on yeast extract glucose agar slopes containing 0.1 per cent calcium carbonate and washed in the usual manner.

The results are given in Table XII. They show that different organisms produced different amounts of acetic acid in a definite length of time and under the same physical conditions. Even equal-sized colonies, made by the same organism, produced clear zones which varied in size.

Experiment 2

The effect was determined of a medium containing 3 per cent ethyl alcohol and 1 per cent casein hydrolysate on the growth

TABLE XII

A STUDY OF THE GROWTH CHARACTERISTICS OF VARIOUS ACETOBACTER SPECIES WITH 2 PER CENT ETHYL ALCOHOL AND YEAST EXTRACT AS SOLE SOURCE OF NITROGEN

Organism		Growth		Zone of clearing (in cm.)													
				2 day				3 day				4 day				9 day			
				I[k]		II[l]		I		II		I		II		I		II	
		2 day	4 day	Col[m]	Cl[n]	Col	Cl	Col	Cl	Col	Cl	Col	Cl	Col	Cl	Col	Cl	Col	Cl
A. peroxydans	T[i]	(≠)[p]	≠[a]	0				0.4	0.7	0.4	1.0	0.4	0.9	0.35	1.25	0.4	1.5	0.5	1.45
	C[j]	(≠)	≠																
A. melanogenum 2	T	≠		0.4	1.2	0.4	2.0	0.4	2.0	0.4	3.1	0.4	2.1	0.4	3.3	0.45	2.45	0.4	Indefinite
	C	≠																	
A. suboxydans	T	≠		0				0.4		0.4		0.4							
	C	0	0																
A. acetosum	T	≠		(≠)				0.4	1.0	0.4	1.6	0.4	0.95	0.45	1.85	0.4	1.85	0.4	2.6
	C	0																	
A. ascendens	T	≠		(≠)				0.4	1.0	0.4	2.0	0.4	1.2	0.45	2.2	0.5	1.70	0.4	2.95
	C	(≠)	≠																
A. pasteurianum	T	≠		(≠)				0.35	0.7	0.4	0.9	0.25	0.8	0.4	1.0	0.4	0.95	0.4	2.1
	C	≠																	
A. turbidans	T	(≠)		0				0				((≠))	-			0.4	1.0	0.4	1.35
	C	0	((≠))[o]																
A. kützingianum	T	(≠)		((≠))				0.4	0.7	0.4	2.0	0.4	1.0	0.4	2.25	0.45	1.6	0.45	2.5
	C	(≠)	≠																
A. aceti 1	T	≠		0.4	0.8	0.4	1.2	0.4	1.5	0.4	2.2	0.4	1.7	0.4	2.4	0.4	2.7	0.5	2.55
	C	≠																	
A. aceti 2	T	≠														0.4	1.6	0.5	2.3
	C	≠																	
A. rancens	T	≠		0.4	0.65	0.4	1.2	0.4	1.5	0.4	2.3	0.45	1.25	0.4	2.65	0.5	1.85	0.5	3.0
	C	≠																	
A. gluconicum	T	≠		((≠))					0.6			0.3	0.3	0.4	0.75	0.3	0.8	0.4	1.3
	C	((≠))	((≠))																
A. melanogenum 1	T	≠	≠	0.4	0.9	0.5	1.5	0.4	1.4	0.4	2.3	0.4	1.45	0.45	2.45	0.4	2.1	0.5	3.6
	C	((≠))	≠																
A. oxydans	T	≠		0.4	1.0	0.4	1.8	0.4	1.85	0.4	2.8	0.4	2.1	0.45	3.15	0.5	2.6	0.5	3.5
	C	≠																	

i - T - Test. j - C - Control. k - I - Diameter of repres. colony with clear zone on plate.
l - II - Diameter of second repres. colony with clear zone on plate. m - Col - Diameter of colony.
n - Cl - Diameter of clear zone produced by colony. o - ((≠)) - Very poor growth. p - (≠) - Poor growth.
a - ≠ - Growth.

characteristics of Acetobacter species. The basic procedure was followed (page 71). The indicated ingredients replaced the yeast extract in the basal medium.

Casein hydrolysate	10.0 ml.
Calcium chloride	0.1 gm.
Ferric chloride	0.2 gm.
Magnesium sulphate	0.2 gm.
Dibasic potassium phosphate	1.0 gm.
Sodium chloride	0.1 gm.

The ingredients were dissolved in distilled water. Three per cent ethyl alcohol was used.

The results are given in Table XIII. Some organisms produced clear zones in the test medium only. One organism grew but did not produce a clear zone. Other organisms did not grow. Acetobacter kützingianum produced a clearing of the calcium carbonate in the control but not in the test medium. Acetobacter oxydans showed variability in clearing the calcium carbonate amongst individual bacteria within a colony. The latter organism was plated, and colonies appearing slightly different were isolated. All succeeding experiments contain the two types of bacteria isolated and they will be referred to as Acetobacter oxydans 1 and Acetobacter oxydans 2.

TABLE XIII

A STUDY OF THE GROWTH CHARACTERISTICS OF VARIOUS ACETOBACTER SPECIES WITH 3 PER CENT ETHYL ALCOHOL AND 1 PER CENT CASEIN HYDROLYSATE AS SOLE SOURCE OF NITROGEN

Organism		Growth		Zone of clearing (in cm.)											
		3 day	8 day	3 day				6 day				8 day			
				I		II		I		II		I		II	
				Col	Cl	Col	Cl	Col	Cl	Col	Cl	Col	Cl	Col	Cl
A. peroxydans	T	≠		0.5	0.9			0.2	1.2	0.3	3.4	0.25	1.85	0.4	3.0
	C	≠													
A. suboxydans	T		0									0			
	C		0									0			
A. acetosum	T		0									0			
	C		0									0			
A. ascendens	T	≠						≠				0.3	0.4		
	C	≠						≠					Sl[q]		
A. pasteurianum	T	≠						≠					Sl		
	C	≠						≠					Sl		
A. turbidans	T	0	(≠)					0				0			
	C	0	(≠)					0				0			
A. kützingianum	T	≠						≠					0		
	C	≠						≠					Sl		
A. aceti 1	T	≠		0.35	1.0	0.3	0.8	0.3	2.0	0.3	1.6	0.35	2.25	0.4	2.85
	C	≠						0				0			
A. aceti 2	T	≠		≠				0.3	0.5			0.3	1.1		
	C	≠											Sl		
A. rancens	T	≠						0				0.3	0.8	0.4	1.1
	C	≠						0							
A. gluconicum	T		0					0				0			
	C		0					0				0			
A. melanogenum 1	T		0									0			
	C		0									0			
A. oxydans	T	(≠)	≠					≠				hetero[r]			
	C	(≠)	≠					≠							

q - Sl - slight clearing.
r - hetero - heterogeneous colonies.

Experiment 3

The effect was determined of a medium containing 3 per cent ethyl alcohol, 1 per cent casein hydrolysate, and growth factors on the growth characteristics of Acetobacter. The basic procedure was followed (page 71). The indicated growth factors were used. Solutions of them were made as follows:

p-Aminobenzoic acid: 50 mg. dissolved in 50 ml. distilled water containing 0.5 ml. glacial acetic acid. Before use the solution was diluted 1:100; i.e., 1 ml. contained 10 µg.

Vitamin B-12: 1 vial dissolved in 150 ml. 20 per cent ethyl alcohol; i.e., 40 ml. contained 4.0 µg.

Biotin: 1 vial dissolved in distilled water and the volume made up to 250 ml.; i.e., 1 ml. contained 0.1 µg.

Folic acid: 1.0 mg. dissolved in 250 ml. distilled water and the volume made up to 500 ml. with 90 per cent ethyl alcohol; i.e., 1 ml. contained 2 µg.

Inositol: 0.625 gm. dissolved in 12.5 ml. distilled water; i.e., 1 ml. contained 0.05 gm.

Nicotinic acid: 5.0 mg. dissolved in 50 ml. distilled water; i.e., 1 ml. contained 100 µg.

Pantothenic acid: 5.0 mg. dissolved in 50 ml. distilled water; i.e., 1 ml. contained 100 µg.

Pyridoxine: 10 mg. dissolved in 50 ml. distilled water; i.e., 1 ml. contained 200 µg.

Riboflavin: 6.0 mg. dissolved in 50 ml. distilled water; i.e., 1 ml. contained 120 µg.

Thiamine: 5.0 mg. dissolved in 50 ml. distilled water; i.e., 1 ml. contained 100 µg.

One milliliter of each, except 40 ml. for vitamin B-12, was used for preparing media. The casein hydrolysate and mineral salts used in Experiment 2, except ferric chloride, and growth factors replaced the yeast extract in the basal medium. In the present and later experiments 0.02 gm. ferric chloride was used. The number of ml. of solutions of the growth factors was calculated in order to determine the amount of distilled water that should be added.

Growth and clearing results are given in Table XIV. The growth results of the organisms were somewhat comparable with those using casein hydrolysate alone (Experiment 2). Clearing results were somewhat similar and again clearing appeared in some of the control plates.

Experiment 4

The effect was determined of a medium containing 3 per cent ethyl alcohol, 0.01 per cent casein hydrolysate and growth factors on the growth characteristics of Acetobacter. An experiment similar to Experiment 3 was set up. Folic acid and vitamin B-12 were omitted. One-tenth milliliter of casein hydrolysate was used. Difco's Micro Assay Culture Agar was used as stock culture medium.

The results, given in Table XV, page 81, show that Acetobacters grew better than in the medium used in Experiment 3. Clearing zones were somewhat better, and were more obvious in the test than in the control plates.

TABLE XIV

A STUDY OF THE GROWTH CHARACTERISTICS OF VARIOUS ACETOBACTER SPECIES WITH 3 PER CENT ETHYL ALCOHOL AND 1 PER CENT CASEIN HYDROLYSATE AS SOLE SOURCE OF NITROGEN IN THE PRESENCE OF GROWTH FACTORS (See experiment 3)

Organism		Growth		Zone of clearing (in cm.)					
		3 day	8 day	3 day I		8 day I		8 day II	
				Col	Cl	Col	Cl	Col	Cl
A. peroxydans	T	≠				0.3	0.85		
	C	≠				0.35	0.7		
A. suboxydans	T		0			0			
	C		0			0			
A. acetosum	T		0			0			
	C		0			0			
A. ascendens	T		(≠)			0	0		
	C		(≠)			0.38	0.8		
A. pasteurianum	T	≠			(≠)		Sl		
	C	≠			(≠)		Sl		
A. turbidans	T		(≠)				0		
	C		(≠)				0		
A. kützingianum	T		((≠))				0		
	C		≠				Sl		
A. aceti 1	T	≠		0.3	0.6	0.3	2.6	0.4	3.3
	C	≠		0.3	1.0	0.3	1.8		
A. aceti 2	T	≠			(≠)		Sl		
	C	≠			(≠)		Sl		
A. rancens	T	(≠)	≠			0.35	0.65		
	C	(≠)	≠		V.Sl[s]	0.35	0.7	0.3	0.8
A. gluconicum	T		0			0			
	C		0			0			
A. melanogenum 1	T		0			0			
	C		0			0			
A. oxydans	T		≠			heter.			
	C		≠			heter.			

s - V.Sl - Very slight clearing.

TABLE XV

A STUDY OF THE GROWTH CHARACTERISTICS OF VARIOUS ACETOBACTER SPECIES WITH 3 PER CENT ETHYL ALCOHOL AND 0.01 PER CENT CASEIN HYDROLYSATE AS SOLE SOURCE OF NITROGEN IN THE PRESENCE OF GROWTH FACTORS

(See Experiment 4)

Organism		Growth			Zone of clearing (in cm.)											
		3 day	5 day	8 day	3 day				5 day				8 day			
					I		II		I		II		I		II	
					Col	Cl	Col	Cl	Col	Cl	Col	Cl	Col	Cl	Col	Cl
A. peroxydans	T	≠				Sl			0.35	0.85	0.3	1.1	0.35	1.6	0.4	2.05
	C	≠				V.Sl				Sl				Sl		
A. suboxydans	T	≠												0		
	C	≠												0		
A. acetosum	T	0														
	C	0														
A. ascendens	T	(≠)				0				V.Sl				Sl		
	C	(≠)				0				V.Sl				V.Sl		
A. pasteurianum	T	≠				Sl			0.4	0.7	0.3	0.5	0.4	0.6	0.35	0.7
	C	≠				V.Sl				V.Sl				V.Sl		
A. turbidans	T	(≠)?[t]		(≠)?										0		
	C	(≠)?		(≠)?										0		
A. kützingianum	T	(≠)		≠		V.Sl				Sl			0.3	0.6	0.25	0.55
	C	(≠)		≠		0				V.Sl				V.Sl		
A. aceti 1	T	≠				Sl			0.3	0.4	0.3	0.45	0.35	0.5	0.3	0.55
	C	≠				V.Sl				Sl				Sl		
A. aceti 2	T	≠				Sl			0.35	0.5	0.2	0.45	0.2	0.5	0.3	0.6
	C	≠				V.Sl				V.Sl				Sl		
A. rancens	T	≠				Sl			0.3	0.4	0.3	0.4	0.3	0.45	0.3	0.45
	C	≠				V.Sl				Sl				Sl		
A. gluconicum	T	0														
	C	0														
A. melanogenum 1	T	(≠)?		≠										Sl		
	C	(≠)?		0												
A. oxydans 1	T	≠				V.Sl				Sl			0.3	0.35	0.3	0.6
	C	≠				V.Sl				V.Sl				V.Sl		
A. oxydans 2	T	(≠)		(≠)		V.Sl								Sl		
	C	(≠)		(≠)		V.Sl								V.Sl		

t - (≠)? - Questionable growth.

Experiment 5

The effect was determined of a medium containing 3 per cent ethyl alcohol and 0.5 per cent Casamino Acids on the growth characteristics of Acetobacter species. An experiment similar to Experiment 2 (page 74) was set up. Five grams of Difco's Casamino Acids was used, however, in place of 10 ml. of casein hydrolysate and 0.02 gm. of ferric chloride in place of 0.2 gm. Yeast extract glucose agar slopes containing 2 per cent calcium carbonate were used for subculturing the Acetobacter species.

The results, Table XVI, compare well with those of the experiment in which casein hydrolysate was used, Table XIII, page 77. The two experiments cannot be compared too rigidly because the ferric chloride concentration was different.

v. Discussion

Further experiments were not done by means of Method II. It served a purpose in detection of oxidation of ethyl alcohol and also of growth of the organism in the test medium. Although the same loop was used for each inoculum and the medium was well mixed before plating, the difference in size of clear zones produced by each organism in the same medium was great. Also, as will be observed with the next method, when tests were set up using a different method and the same medium, the two sets of results were not sufficiently comparable.

TABLE XVI

A STUDY OF THE GROWTH CHARACTERISTICS OF VARIOUS ACETOBACTER SPECIES WITH 3 PER CENT ETHYL ALCOHOL AND 0.5 PER CENT CASAMINO ACIDS AS SOLE SOURCE OF NITROGEN

Organism		Growth			Zone of clearing (in cm.)											
					4 day				6 day				8 day			
					I		II		I		II		I		II	
		4 day	6 day	8 day	Col	Cl	Col	Cl	Col	Cl	Col	Cl	Col	Cl	Col	Cl
A. peroxydans	T	≠			0.35	0.6			0.35	1.4	0.4	1.5	0.4	2.0	0.4	1.85
	C	≠				0				0				0		
A. suboxydans	T	(≠)	(≠)	(≠)										0		
	C	(≠)?		0										0		
A. acetosum	T		(≠)	≠										0		
	C	≠												0		
A. ascendens	T		(≠)	≠						0				Sl		
	C		(≠)	≠						0				0		
A. pasteurianum	T	≠				Sl			0.45	0.7	0.3	0.6	0.3	0.7	0.45	0.8
	C	≠				V.Sl				Sl			0.4	0.6	0.4	0.6
A. turbidans	T	≠				V.Sl								Sl		
	C	≠				V.Sl								V.Sl		
A. kützingianum	T	≠				0			0.4	0.55		V.Sl	0.4	1.3	0.3	1.0
	C	≠				0			0.45	0.6	0.3	0.55	0.35	0.5	0.4	0.5
A. aceti 1	T	≠			0.4	1.35	0.4	1.5	0.35	2.3	0.35	2.2	0.4	2.45	0.4	2.35
	C	≠				0				0				0		
A. aceti 2	T	≠				Sl				Sl			0.45	0.65	0.4	0.7
	C	≠				V.Sl				V.Sl				V.Sl		
A. rancens	T	≠				Sl			0.35	0.55	0.3	0.55	0.3	0.95	0.4	1.05
	C	≠				0				0				0		
A. gluconicum	T			0										0		
	C			0										0		
A. melanogenum 1	T			0										0		
	C			0										0		
A. oxydans 1	T	≠				Sl			0.35	0.95	0.45	1.1	0.35	1.85	0.35	1.5
	C	≠				0				0				0		
A. oxydans 2	T	≠				V.Sl			0.3	0.65	0.2	0.55	0.25	1.15	0.35	1.45
	C	≠				0				V.Sl				V.Sl		

d. Method III

. Adaptation of Erlenmeyer Flasks to Simulate Conditions of the "Orleans" Process

i. Procedure

Media of varying composition were put into Erlenmeyer flasks and inoculated with various Acetobacter organisms. On removal of each 10 ml. for titration with 0.1 normal sodium hydroxide, 10 ml. sterile medium was added. The flask was a substitution for the barrel in the "slow" vinegar process. Various modifications, as will be described, were attempted to increase the acetic acid yield. One milliliter of a suspension of each organism, prepared as described on page 51, was added to each flask of medium. In several instances the ingredients of the medium were the same as those used in Method II. Calcium carbonate and agar, however, were omitted. Each of the parallel tests will be clearly indicated.

ii. A Study of Variations in the Method and Acetic Acid Production by Acetobacter Organisms in Various Nutrient Media

Experiment 1

The effect was determined of increased aeration on acetic acid production by Acetobacter suboxydans. One set of three 1000 ml. flasks containing 200 ml. of the following medium was set up:

Yeast extract	1 gm.
Magnesium sulphate	2 gm.
Dibasic potassium phosphate	3 gm.
Sodium chloride	2 gm.
Tap water	930 ml.

The above nutrients were dissolved in tap water, autoclaved, made up to 1000 ml. volume containing 7 per cent 95 per cent ethyl alcohol and distributed into sterile 1000 ml. flasks. Two flasks were inoculated and all were placed on a shaker to facilitate greater aeration.

Titration readings are given in Table XVII. The experiment was done while doing the preliminary experiments by means of the perfusion method. The results obtained were not as good as those recorded for a similar test using that method (Table V, page 58). The procedure was, therefore, discontinued.

Experiment 2

Acetic acid production by Acetobacter species from 3 per cent ethyl alcohol was determined in a medium containing yeast extract as sole source of nitrogen. A one per cent solution of yeast extract was made with water, and adjusted to a pH of 6.0. A sufficient quantity of the medium was autoclaved. Three per cent of 95 per cent ethyl alcohol was added to the medium that was to be used in test flasks, but not to the medium that was to be used in control flasks. Approximately 100 ml. quantities were placed in 250 ml. Erlenmeyer flasks so that one test and one control medium were studied for each

TABLE XVII

ACID PRODUCTION FROM 7 PER CENT ETHYL ALCOHOL BY *ACETOBACTER SUBOXYDANS* IN A MEDIUM CONTAINING YEAST EXTRACT AS SOLE SOURCE OF NITROGEN IN A KAHN SHAKER TO FACILITATE AERATION, TITRATION READINGS, ML.

Time days	Test 1	Test 2	Control
1	1.60	1.60	1.60
2	1.80	1.90	1.60
3	2.10	2.40	1.60
5	2.80	3.20	1.60
6	3.60	4.00	1.60
8	4.20	4.50	1.65
11	4.35	4.80	1.60

organism. One milliliter of a suspension of each organism, prepared in the usual way, was added to one test flask,also to one control flask. Titrations of 10 ml. quantities were made with 0.1 normal sodium hydroxide at three, five and eight-day intervals.

The results are recorded in Table XVIII, and a comparison of the results with those from a similar test using the "plate" method is given in Table XIX, page 89. The readings indicate that in the nutritive medium used, different species of Acetobacter varied in their ability to produce acetic acid from ethyl alcohol. The results obtained using the flasks, that is the "slow" vinegar process, did not compare well with those using calcium carbonate plates. The former method seemed more sensitive.

Experiment 3

The effect was determined of a medium containing 3 per cent ethyl alcohol and 1 per cent casein hydrolysate on acetic acid production by Acetobacter. A medium was prepared containing casein hydrolysate and the basic salts as described in Method II, Experiment 2, on page 74. Ninety-seven milliliters of medium were put into a sufficient number of flasks to have a test and a control for each organism. The flasks were autoclaved and 3 ml. of 95 per cent ethyl alcohol were added to the test flasks. The flasks of each set were inoculated with 1 ml. of a suspension of each organism.

TABLE XVIII

ACETIC ACID PRODUCTION FROM 3 PER CENT ETHYL ALCOHOL WITH YEAST EXTRACT AS SOLE SOURCE OF NITROGEN AND VARIOUS ACETOBACTER SPECIES AS TEST BACTERIA

Organism		Readings					
		3 day		5 day		8 day	
		Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm
A. peroxydans	T	0.85	0.012	1.20	0.045	1.65	0.087
	C	0.65		0.45		0.20	
A. melanogenum 2	T	27.85	1.632	24.45	1.422	36.40	2.178
	C	0.65		0.75		0.10	
A. suboxydans	T	0.80	0.015	0.55		0.80	0.027
	C	0.55		0.65		0.35	
A. acetosum	T	1.30	0.048	3.25	0.174	28.30	1.698
	C	0.50		0.35		0.00	
A. ascendens	T	0.95	0.030	2.25	0.111	25.40	1.518
	C	0.45		0.40		0.10	
A. pasteurianum	T	0.95	0.027	1.35	0.078	1.80	0.108
	C	0.50		0.05		0.00	
A. turbidans	T			0.65	0.018	0.80	0.036
	C	0.50		0.35		0.20	
A. kützingianum	T	1.00	0.03	1.70	0.084	12.75	0.756
	C	0.50		0.30		0.15	
A. aceti 1	T	4.65	0.279	19.15	1.149	28.55	1.713
	C	0.00		0.00		0.00	
A. aceti 2	T	5.20	0.282	26.60	1.590	27.65	1.659
	C	0.50		0.10		0.00	
A. rancens	T	10.60	0.618	41.80	2.490	22.55	1.347
	C	0.30		0.30		0.10	
A. gluconicum	T	0.70	0.006	0.50		1.35	0.063
	C	0.60		1.05		0.30	
A. melanogenum 1	T	1.70	0.069	2.55	0.129	3.85	0.213
	C	0.55		0.40		0.30	
A. oxydans	T	12.05	0.705	42.05	2.511	40.00	2.391
	C	0.30		0.20		0.15	
A. xylinum	T	0.70	0.003	0.90	0.012	0.35	
	C	0.65		0.70		0.50	

TABLE XIX

ORGANISMS LISTED IN THE ORDER IN WHICH THEY PRODUCED ACETIC ACID IN THE YEAST EXTRACT MEDIUM CONTAINING 3 PER CENT ETHYL ALCOHOL BY THE PLATE AND FLASK METHODS

(The largest clearing diameter in centimeters and the greatest titration of N/10 NaOH in milliliters with 10 ml. of broth are indicated for each organism)

Calcium carbonate plates		Flasks containing broth	
Organism	Largest clearing diameter, cm	Organism	Greatest titration with N/10 NaOH, ml
A. melanogenum 2	indefinite	A. oxydans	41.85
A. melanogenum 1	3.60	A. rancens	41.50
A. oxydans	3.50	A. melanogenum 2	36.30
A. rancens	3.00	A. aceti 1	28.55
A. ascendens	2.95	A. acetosum	28.30
A. acetosum	2.60	A. aceti 2	27.65
A. aceti 1	2.55	A. ascendens	25.30
A. kützingianum	2.50	A. kützingianum	12.60
A. aceti 2	2.30	A. melanogenum 1	3.55
A. pasteurianum	2.10	A. pasteurianum	1.80
A. peroxydans	1.50	A. peroxydans	1.45
A. turbidans	1.35	A. gluconicum	1.05
A. gluconicum	1.30	A. turbidans	0.60
A. suboxydans	0.00	A. suboxydans	0.45

The results are recorded in Table XX, and a comparison of the results with those from a similar test using the "plate" method is given in Table XXI, page 92. The results indicate that some species of Acetobacter produced a small quantity of acetic acid when grown in a casein hydrolysate medium. Other nutrients were, however, essential. "Plate" and "flask" method readings were different.

Experiment 4

The effect was determined of a medium containing 3 per cent ethyl alcohol, 1 per cent casein hydrolysate and growth factors on acetic acid production by Acetobacter species. An experiment was set up using the method of Experiment 3 and the ingredients in Method II, Experiment 3, page 78.

The results are given in Table XXII, page 93. A comparison of the results with those from a similar test using the "plate" method is given in Table XXIII, page 94. The most striking factor observed by each method is that more acid was sometimes produced when ethyl alcohol was not present in the medium. The results indicate that the readings taken from the "plate" and "flask" methods were not comparable.

TABLE XX

ACETIC ACID PRODUCTION FROM 3 PER CENT ETHYL ALCOHOL WITH 1 PER CENT CASEIN HYDROLYSATE AS SOLE SOURCE OF NITROGEN AND VARIOUS ACETOBACTER SPECIES AS TEST BACTERIA

Organism		Readings					
		3 day		5 day		8 day	
		Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm
A. peroxydans	T	0.90	0.018			1.65	0.063
	C	0.60				0.60	
A. suboxydans	T	0.60	0.000	0.60	0.000	0.70	0.009
	C	0.60		0.60		0.55	
A. acetosum	T	0.75	0.009	0.80	0.015	0.95	0.021
	C	0.60		0.55		0.60	
A. ascendens	T	0.70	0.009	0.80	0.015	0.80	0.021
	C	0.55		0.55		0.60	
A. pasteurianum	T	0.90	0.018	1.20	0.036	1.40	0.048
	C	0.60		0.60		0.60	
A. turbidans	T	0.60	0.000	0.85	0.018	0.85	0.024
	C	0.60		0.55		0.45	
A. kützingianum	T	0.85	0.015	1.00	0.027	4.10	0.216
	C	0.60		0.55		0.50	
A. aceti 1	T	1.10	0.033	1.35	0.048	1.65	0.066
	C	0.55		0.55		0.55	
A. aceti 2	T	0.85	0.015	1.00	0.024	1.05	0.027
	C	0.60		0.60		0.60	
A. rancens	T	0.80	0.018	1.10	0.039	1.50	0.060
	C	0.50		0.45		0.50	
A. gluconicum	T	0.60	0.003	0.60	0.000	0.60	0.000
	C	0.55		0.60		0.60	
A. melanogenum 1	T	0.70	0.006	0.80	0.012	0.75	0.006
	C	0.60		0.60		0.65	
A. oxydans	T	1.10	0.030	1.35	0.051	1.75	0.078
	C	0.60		0.50		0.45	

TABLE XXI

ORGANISMS LISTED IN THE ORDER IN WHICH THEY PRODUCED ACETIC ACID IN A 1 PER CENT CASEIN HYDROLYSATE MEDIUM CONTAINING 3 PER CENT ETHYL ALCOHOL BY THE PLATE AND THE FLASK METHODS

(The greatest clearing diameter in centimeters and the greatest titration of N/10 sodium hydroxide against 10 ml. of broth are indicated for each organism)

Calcium carbonate plates		Flasks containing broth	
Organism	Largest clearing diameter, cm	Organism	Greatest titration with N/10 NaOH, ml
A. peroxydans	3.00	A. kützingianum	3.60
A. aceti 1	2.85	A. oxydans	1.30
A. rancens	1.10	A. aceti 1	1.10
A. aceti 2	1.10	A. peroxydans	1.05
A. ascendens	0.40	A. rancens	1.00
A. oxydans	Sl	A. pasteurianum	0.80
A. suboxydans	0.00	A. aceti 2	0.45
A. acetosum	0.00	A. turbidans	0.40
A. pasteurianum	0.00	A. acetosum	0.35
A. turbidans	0.00	A. ascendens	0.20
A. gluconicum	0.00	A. suboxydans	0.15
A. melanogenum 1	0.00	A. melanogenum 1	0.10
A. kützingianum	Sl in control	A. gluconicum	0.00

TABLE XXII

ACETIC ACID PRODUCTION FROM 3 PER CENT ETHYL ALCOHOL WITH 1 PER CENT CASEIN HYDROLYSATE AS SOLE SOURCE OF NITROGEN IN THE PRESENCE OF THE GROWTH FACTORS AND VARIOUS ACETOBACTER SPECIES AS TEST BACTERIA

Organism		Readings					
		3 day		5 day		8 day	
		Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm
A. peroxydans	T	0.75		1.10		1.80	
	C	0.85		1.15		1.95	
A. suboxydans	T	0.70	0.006	0.70	0.003	0.70	0.003
	C	0.60		0.65		0.65	
A. acetosum	T	0.70	0.000	0.85	0.003	0.85	0.003
	C	0.70		0.80		0.80	
A. ascendens	T	0.70		0.70		0.75	
	C	0.75		0.80		0.85	
A. pasteurianum	T	0.95	0.000	1.10	0.000	1.25	0.003
	C	0.95		1.10		1.20	
A. turbidans	T	0.75	0.000	0.75		0.85	
	C	0.75		0.85		0.95	
A. kützingianum	T	0.70	0.000	0.90		1.15	
	C	0.70		1.00		1.875	
A. aceti 1	T	1.05		1.30		1.75	
	C	1.25		1.55		2.10	
A. aceti 2	T	0.75		0.75		0.70	
	C	0.80		0.80		0.85	
A. rancens	T	0.75		0.85	0.000	0.95	
	C	0.80		0.85		1.15	
A. gluconicum	T	0.70	0.000	0.65	0.000	0.70	0.000
	C	0.70		0.65		0.70	
A. melanogenum 1	T	0.70		0.70		0.75	
	C	0.75		0.85		0.95	
A. oxydans	T	0.70		0.80		1.25	
	C	0.75		0.95		1.70	

TABLE XXIII

ORGANISMS LISTED IN THE ORDER IN WHICH THEY PRODUCED ACETIC ACID IN A 1 PER CENT CASEIN HYDROLYSATE AND GROWTH FACTOR MEDIUM CONTAINING 3 PER CENT ETHYL ALCOHOL BY THE PLATE AND FLASK METHODS

(The greatest clearing diameter in centimeters and the greatest titration of N/10 sodium hydroxide against 10 ml. of broth are indicated for each organism)

Calcium carbonate plates		Flasks containing broth	
Organism	Largest clearing diameter, cm	Organism	Greatest titration with N/10 NaOH, ml
A. aceti 1	1.50	A. suboxydans	0.05
A. peroxydans	0.15	A. acetosum	0.05
A. suboxydans	0.00	A. pasteurianum	0.05
A. acetosum	0.00	A. gluconicum	0.00
A. turbidans	0.00	A. kützingianum	0.725[v] in control
A. gluconicum	0.00	A. oxydans	0.45 in control
A. melanogenum 1	0.00	A. aceti 1	0.35 in control
A. oxydans	0.00	A. rancens	0.20 in control
A. aceti 2	0.00	A. melanogenum 1	0.20 in control
A. pasteurianum	0.00	A. peroxydans	0.15 in control
A. ascendens	0.80[u] in control	A. aceti 2	0.15 in control
A. rancens	0.15 in control	A. ascendens	0.10 in control
A. kützingianum	V.Sl in control	A. turbidans	0.10 in control

u - 0.80 in control - means the diameter of the clear zone in the control is 0.80 cm. larger than that in the test.

v - 0.725 in control - means the titration of the control sample is 0.725 ml. greater than that of the test.

Experiment 5

The effect was determined of a medium containing 3 per cent ethyl alcohol, 0.01 per cent casein hydrolysate and growth factors on acetic acid production by Acetobacter species. An experiment was set up using the method of Experiment 3 and the ingredients of Method II, Experiment 4, page 79.

The results are given in Table XXIV. A comparison of the results with those from a similar test using the "plate" method is given in Table XXV, page 97. Although acid production was very slight, acid was not produced in greater quantity in the control flasks than in the test. Again the readings taken by the "flask" method did not compare well with those taken by the "plate" method.

Experiment 6

The effect was determined of a medium containing 3 per cent ethyl alcohol and 0.5 per cent Casamino Acids on acetic acid production by Acetobacter species. An experiment was set up using the method of Experiment 3 and the ingredients of Method II, Experiment 5, page 82.

The results are given in Table XXVI, page 98. A comparison of the results with those from a similar test using the "plate" method is given in Table XXVII, page 99. The results indicate that acetic acid was produced only in a very small amount. The results were somewhat similar to those obtained with the

TABLE XXIV

ACETIC ACID PRODUCTION FROM 3 PER CENT ETHYL ALCOHOL WITH 0.01 PER CENT CASEIN HYDROLYSATE AS SOLE SOURCE OF NITROGEN IN THE PRESENCE OF GROWTH FACTORS AND VARIOUS ACETOBACTER SPECIES AS TEST BACTERIA

Organism		Readings					
		3 day		5 day		8 day	
		Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm
A. peroxydans	T	0.500	0.0015	0.650	0.009	0.70	0.012
	C	0.475		0.500		0.50	
A. suboxydans	T	0.500		0.500	0.000	0.55	0.000
	C	0.525		0.500		0.55	
A. acetosum	T	0.550	0.0060	0.550	0.003	0.50	0.003
	C	0.450		0.500		0.45	
A. ascendens	T	0.600	0.0060	0.625	0.009	0.65	0.009
	C	0.500		0.475		0.50	
A. pasteurianum	T	0.750	0.0120	0.950	0.027	1.30	0.048
	C	0.550		0.500		0.50	
A. turbidans	T	0.750	0.015	0.925	0.027	1.00	0.030
	C	0.500		0.475		0.50	
A. kützingianum	T	0.700	0.015	0.800	0.078	1.00	0.030
	C	0.450		0.500		0.50	
A. aceti 1	T	0.600	0.006	0.650	0.009	0.65	0.009
	C	0.500		0.500		0.50	
A. aceti 2	T	0.500	0.000	0.550	0.009	0.55	0.003
	C	0.500		0.400		0.50	
A. rancens	T	0.650	0.009	0.750	0.015	0.85	0.024
	C	0.500		0.500		0.45	
A. gluconicum	T	0.500		0.500	0.000	0.50	0.000
	C	0.550		0.500		0.50	
A. melanogenum 1	T	0.650	0.006			0.80	0.015
	C	0.550				0.55	
A. oxydans 1	T	0.750	0.0165	0.850	0.021	1.05	0.030
	C	0.475		0.500		0.55	
A. oxydans 2	T	0.500		0.675	0.012	0.75	0.015
	C	0.550		0.475		0.50	

TABLE XXV

ORGANISMS LISTED IN THE ORDER IN WHICH THEY PRODUCED ACETIC ACID IN A 0.01 PER CENT CASEIN HYDROLYSATE + GROWTH FACTOR MEDIUM CONTAINING 3 PER CENT ETHYL ALCOHOL BY THE PLATE AND FLASK METHODS

(The greatest clearing diameter in centimeters and the greatest titration of N/10 sodium hydroxide against 10 ml. of broth are indicated for each organism)

Calcium carbonate plates		Flasks containing broth	
Organism	Largest clearing diameter, cm.	Organism	Greatest titration with N/10 NaOH, ml
A. peroxydans	2.05	A. pasteurianum	0.80
A. pasteurianum	0.70	A. oxydans 1	0.50
A. oxydans 1	0.60	A. turbidans	0.50
A. aceti 2	0.60	A. kützingianum	0.50
A. kützingianum	0.55	A. rancens	0.40
A. aceti 1	0.55	A. melanogenum 1	0.25
A. rancens	0.45	A. oxydans 2	0.25
A. melanogenum 1	Sl	A. peroxydans	0.20
A. ascendens	V.Sl	A. ascendens	0.15
A. oxydans 2	V.Sl	A. aceti 1	0.15
A. suboxydans	0.00	A. acetosum	0.05
A. acetosum	0.00	A. aceti 2	0.05
A. turbidans	0.00	A. suboxydans	0.00
A. gluconicum	0.00	A. gluconicum	0.00

TABLE XXVI

ACETIC ACID PRODUCTION FROM 3 PER CENT ETHYL ALCOHOL WITH 0.5 PER CENT CASAMINO ACIDS AS SOLE SOURCE OF NITROGEN AND VARIOUS *ACETOBACTER* SPECIES AS TEST BACTERIA

Organism		Readings					
		4 day		5 day		8 day	
		Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm
A. peroxydans	T	1.10		1.450	0.045	1.85	0.072
	C			0.700		0.65	
A. suboxydans	T	0.75		0.750	0.006	0.80	0.006
	C			0.650		0.70	
A. acetosum	T	1.05		1.350	0.042	1.75	0.066
	C			0.650		0.65	
A. ascendens	T	0.90		1.150	0.027	1.35	0.042
	C			0.700		0.65	
A. pasteurianum	T	1.20		1.400	0.042	1.70	0.063
	C			0.700		0.65	
A. turbidans	T	1.15		1.425	0.042	1.65	0.057
	C			0.725		0.70	
A. kützingianum	T	1.15		1.450	0.045	1.50	0.054
	C			0.700		0.60	
A. aceti 1	T	1.85		2.250	0.096	4.85	0.252
	C			0.650		0.650	
A. aceti 2	T	1.20		1.350	0.045	1.50	0.054
	C			0.600		0.60	
A. rancens	T	0.50		0.500	0.000	0.50	0.000
	C			0.500		0.50	
A. gluconicum	T	0.75		0.750	0.003	0.80	0.006
	C			0.700		0.70	
A. melanogenum 1	T	1.10		1.400	0.042	1.650	0.054
	C			0.700		0.750	
A. oxydans 1	T	1.30		1.700	0.066	2.15	0.093
	C			0.600		0.60	
A. oxydans 2	T	0.95		1.450	0.054	2.10	0.093
	C			0.550		0.55	

TABLE XXVII

ORGANISMS LISTED IN THE ORDER IN WHICH THEY PRODUCED ACETIC ACID IN A 0.5 PER CENT CASAMINO ACIDS MEDIUM CONTAINING 3 PER CENT ETHYL ALCOHOL BY THE PLATE AND FLASK METHODS

(The greatest clearing diameter in centimeters and the greatest titration of N/10 sodium hydroxide against 10 ml. of broth are indicated for each organism)

Calcium carbonate plates		Flask containing broth	
Organism	Largest clearing diameter, cm	Organism	Greatest titration with N/10 NaOH, ml
A. aceti 1	2.45	A. aceti 1	4.20
A. peroxydans	2.00	A. oxydans 1	2.15
A. oxydans 1	1.85	A. oxydans 2	1.55
A. oxydans 2	1.45	A. peroxydans	1.20
A. rancens	1.05	A. acetosum	1.10
A. kützingianum	0.70	A. pasteurianum	1.05
A. aceti 2	0.70	A. turbidans	0.95
A. pasteurianum	0.20	A. kützingianum	0.90
A. ascendens	Sl	A. melanogenum 1	0.90
A. turbidans	V.Sl	A. aceti 2	0.90
A. suboxydans	0.00	A. ascendens	0.70
A. acetosum	0.00	A. suboxydans	0.10
A. gluconicum	0.00	A. gluconicum	0.10
A. melanogenum 1	0.00	A. rancens	0.00

medium containing casein hydrolysate, but in the latter experiment a much higher concentration of ferric chloride was used. The readings from the "flask" method did not correspond well with those from the "plate" method.

iii. Discussion

The readings indicated in comparison tables lead to the conclusion that the "flask" method gives a quantitatively accurate test. Where high acetic acid yields were demonstrated, as with specific organisms in a yeast extract medium, comparatively larger clear zoneswere not produced on the calcium carbonate plates. Also, the graded listing of organisms as to their activity in the two sets of results, as given in comparison tables,is not similar. Various sizes of clear zones were produced even by the same organism on the same plate.

Two of the three methods could be used to advantage. If the physical and nutritional factors essential to acetic acid production of Acetobacter were to be studied in large numbers and in detail, it appears that the "flask" method could best serve the purpose. Once a perfect combination of factors could be obtained, however, the apparatus designed by Audus could be used to observe if that result could be improved by the "quick" vinegar process. The following set of experiments is an attempt to standardize the "slow" vinegar process.

iv. An Attempt to Increase Acetic Acid Production by an Increase in the Surface Area within the Flasks

Experiment 1

Cellulose sponges were added to media used during fermentation in order to increase the surface area and thus possibly increase the rate of oxidation of ethyl alcohol by Acetobacter species. A medium of the following content was used:

Yeast extract	10 gm.
Ethyl alcohol	30 ml.
Tap water	970 ml.

The solution of yeast extract was prepared at a pH of 6.0 and added in 97 ml. quantities to several 250 ml. flasks. A piece of cellulose sponge with a 3.8 x 3.8 cm. surface and cut as thin as possible was added to each flask. After autoclaving the medium all sponges had descended to the bottom of the flasks, probably due to the evacuation of the air present in the sponges. The experiment was, therefore, discontinued.

Experiment 2

Cellulose sponges were added to media in another manner in order that they would not descend to the bottom of the flasks. Four 250 ml. flasks of media were prepared as described in Experiment 1. Ethyl alcohol was added after sterilization. Four pieces of sponge were cut to have the largest size surface area which could pass easily through the

mouth of 250 ml. flasks. Sponges of the following dimensions proved successful:

Surface	3.5 x 1.5 cm.
Thickness	0.7 cm.

The pieces of sponge were placed in a Petri dish and autoclaved with the flasks of medium at 15 lb. pressure for fifteen minutes. When the flasks were cool, a piece of sponge was added to the medium in each flask aseptically. Flasks were prepared as indicated below:

Flask 1 - Sterile medium + sterile sponge

Flask 2 - Sterile medium + 3 ml. 95 per cent ethyl alcohol + sterile sponge

Flask 3 - Sterile medium + 3 ml. 95 per cent ethyl alcohol + sterile sponge + Acetobacter ascendens

Flask 4 - Sterile medium + 3 ml. 95 per cent ethyl alcohol + sterile sponge + Acetobacter ascendens.

The organism was transferred with an inoculating needle from a yeast extract glucose agar slope containing 2 per cent calcium carbonate to the broth. The sponges fell to the bottom of flasks 3 and 4, indicating that an evacuation of air had again taken place within the flasks. The experiment was, therefore, discontinued.

Experiment 3

A. Experiment 2 was repeated using cork instead of sponge to increase the surface area. Number 8 corks were cut as follows and the pieces were used:

1. One-half corks cut vertically
2. One-eighth corks cut vertically
3. One-sixteenth corks cut vertically
4. One-eighth corks cut horizontally
5. One-sixteenth corks cut horizontally.

Twelve flasks, containing the medium described in Experiment 1, were used. Three milliliters 95 per cent ethyl alcohol was added to each flask. One part of one type of cork was added to each of two flasks. Corks were not added to two flasks as the latter were controls. Each flask was then inoculated with Acetobacter ascendens.

Titration readings are recorded in Table XXVIII. The control results were as good as the test results.

B. The ability was determined of the corks to float on the surface of the liquid during autoclaving. Five parts of cork, one of each type, were placed each in a separate 250 ml. flask containing 100 ml. tap water. The flasks were sterilized in the usual way. After autoclaving all pieces of cork were still floating but the water had become slightly brown. The latter factor must have been due to the presence in the water of some substance from the cork going into solution, as all the pieces of cork had been thoroughly washed in

TABLE XXVIII

ACID PRODUCTION IN A 1 PER CENT YEAST EXTRACT BROTH MEDIUM CONTAINING 3 PER CENT ETHYL ALCOHOL WITH CORKS OF VARIOUS SIZES TO INCREASE THE SURFACE AREA FOR AERATION

Support for bacterial film		Readings	
		3 day Titration, ml	8 day Titration, ml
Cork type 1	Test 1	3.00	
	2	3.55	
Cork type 2	Test 1	4.00	
	2	3.75	
Cork type 3	Test 1	2.40	
	2	3.60	
Cork type 4	Test 1	3.15	
	2	3.35	
Cork type 5	Test 1	4.05	40.60
	2	3.00	41.30
	Control 1	4.80	40.85
	2	3.00	40.50

detergent, rinsed and dried in the oven.

Since the control results were as good as the test and the cork liberated some substance into the water, the procedure was discontinued.

Experiment 4

The ability was determined of a support to increase the amount of acid produced by facilitating haze formation. Four 250 ml. flasks of medium were set up as described in Experiment 1. A wire, extending from the outside of the flask and forming a large loop on the surface of the medium, was added to each of two flasks. The other two flasks were controls. All flasks were autoclaved, 3 ml. 95 per cent ethyl alcohol was added to each flask and Acetobacter ascendens was used as inoculum.

Titration readings are given in Table XXIX. The readings indicate that the wire, by giving a surface onto which any haze or pellicle can begin formation, did not hasten or increase in quantity the acetic acid formed.

e. Conclusion

For an efficient study of acetic acid production with numerous acetic acid bacteria the "Erlenmeyer flask" method was the most appropriate. Inert material on the surface of the broth in the flasks could not help the oxidation of

TABLE XXIX

ACID PRODUCTION IN A 1 PER CENT YEAST EXTRACT BROTH MEDIUM CONTAINING 3 PER CENT ETHYL ALCOHOL WITH A WIRE ON THE SURFACE OF THE BROTH TO FACILITATE HAZE FORMATION

Support for bacterial film		Readings	
		3 day Titration, ml	8 day Titration, ml
Test	1	3.40	40.55
	2	5.90	40.50
Control	1	4.45	40.25
	2	5.70	40.80

ethyl alcohol by Acetobacter. In summary, 97 ml. of appropriate broth medium were put into 250 ml. flasks and autoclaved. Three milliliters of 95 per cent ethyl alcohol were added to the sterile broth for test but not for control media. One milliliter of a washed suspension of the organism to be used in the test, page 51, was added to each flask. Ten milliliter quantities were taken from each flask at three, five and eight-day intervals and titrated against 0.1 normal sodium hydroxide. Ten milliliters of similar sterile medium was replaced in each flask.

4. Summary

1. All organisms grew well on a medium containing 1 per cent yeast extract (Difco), 1 per cent glucose, 2 per cent calcium carbonate and 1.5 per cent agar.
2. The oxidation of ethyl alcohol to principally acetic acid was the most important characteristic in differentiating the Acetobacter genus.
3. Acetobacter species from apples multiplied in malt extract broth containing 3 per cent ethyl alcohol, and could be isolated on yeast extract glucose agar plates.
4. The method described by Breed et al (5), which appeared to be the only feasible one for studying classification of the genus at present, was used.
5. The perfusion apparatus designed by Audus was adapted to suit the study of acetic acid production by specific Acetobacter species by the "quick" process on laboratory scale. The method

was quantitatively accurate, but required physical conditions which could not always be provided.

6. The calcium carbonate clearing method described served the purpose of detecting growth of Acetobacter in different nutrient media. The method was not in general reliable for detecting acetic acid production.

7. The Erlenmeyer flask was adapted to suit the study of acetic acid production by the "Orleans" process. The process was good for the study of the effect of numerous changes in physical and chemical factors on acid production by numerous species. The method was quantitatively accurate.

E. METHODS

1. Procedure

a. Generalization

For the study of the effect of each variation of a physical or chemical factor on acetic acid production, conditions were maintained generally as described in the adapted "Orleans" process, page 84.

Two sterile alcohol-containing, and one sterile alcohol-omitted media, prepared in 100 ml. amounts in 250 ml. Erlenmeyer flasks, were inoculated with each organism to study separately every variation of the factor being studied. The flasks containing the former media were referred to as "test flasks", as the acid produced during the fermentation of the alcohol-containing media was primarily acetic acid, although small amounts of other acids could have been formed from other components of the media. The flasks containing the latter media were referred to as "control flasks", as the acids produced during the fermentation of the alcohol-omitted media were formed from components other than ethyl alcohol. Later as will be indicated three "test flasks" and one "control flask" were prepared for each organism, because similar fermentations sometimes proceded at greatly varying rates. Three "test flasks", therefore

gave better averages of the rate of acetic acid production.

Ten milliliter samples were taken from test and control flasks at three, five and eight-day intervals during fermentation, and were titrated with 0.1 normal sodium hydroxide, using phenolphthalein as indicator, to determine the amount of acid present. Sufficient appropriate media were made in large quantities so that 10 ml. of a similar sterile broth could be added to the flasks. Later, as will be indicated, samples were taken in the same manner at two, four, six, eight and ten-day intervals. Eleven or twelve-day samples replaced ten-day samples on several occasions due to unforeseen circumstances. The eleven or twelve-day sample titrations were allowed to replace the ten-day, due to possible exhaustion of the nutrients, and the evaporation of the ethyl alcohol and acetic acid in the media in the extra time. However, the appropriate ten-day readings were also obtained through figures and will be referred to.

b. Modification of Procedure

During the final experiment, as will be indicated, the Fisher Titrimeter was used instead of phenolphthalein indicator to determine the amount of acid present in samples. The control flasks consisted of similar sterile alcohol-containing media. Each 10 ml. sample of control

medium was mixed with 70 ml. of distilled water and a titration curve of the pH (ordinate) versus the volume of titrant added (abscissa) was plotted over the range from the pH of the original solution to the pH of the titrant, in order to determine the equivalence point, the point of inflection on the curve. The pH at the equivalence point represented the pH to which inoculated samples of the same type of medium would be titrated on appropriate days, and the quantity of titrant required to obtain the equivalence point of the sterile medium represented the amount of acid in the control flask.

c. Preparation of Inocula

The inocula were prepared by growing each Acetobacter organism on a single straight streak on culture medium for two days and washing the organism as described on page 51. Each appropriate test flask and control was inoculated with 1 ml. of the final suspension of an organism. Changes in procedure will be indicated. A sterile slope of culture medium was inoculated with one loopful of the final suspension of each organism to test the viability of the organism.

d. Determination of the Amount of Acetic Acid

The amount of acetic acid was calculated from the amount of 0.1 normal sodium hydroxide required to neutralize 10 ml. of the fermenting medium as described on page 52.

2. Organisms Applied

The effect of varying physical and chemical factors on acetic acid production was studied with the following organisms of the previous section (page 49):

Acetobacter suboxydans

Acetobacter acetosum

Acetobacter ascendens

Acetobacter pasteurianum

Acetobacter turbidans

Acetobacter kützingianum

Acetobacter aceti 1

Acetobacter aceti 2

Acetobacter rancens

Acetobacter gluconicum

Acetobacter melanogenum 1

Acetobacter oxydans 1

Acetobacter oxydans 2

3. Preparation of Media

a. Source of Nutrients

For the experimental work the substances listed were used. A substance from a different industrial laboratory sometimes had to be substituted during experiments. Changes will be noted in the appropriate experiments.

Complex substances	Source
Bacto-Yeast Extract	Difco Laboratories Incorporated, Detroit 1, Michigan
Yeast Extract (powder)	Nutritional Biochemicals Corporation, Cleveland, Ohio
Amino acids	
Bacto-Vitamin Free Casamino Acids	Difco Laboratories Incorporated
l(+) Cysteine hydrochloride	a Merck and Co. Inc., Rahway, N.J. b Eastman Kodak Company, Rochester,N.Y.
DL Norleucine	Nutritional Biochemicals Corporation
l-Tryptophane	a Difco Laboratories Incorporated b Nutritional Biochemicals Corporation
Purine bases	
Adenine sulphate	Nutritional Biochemicals Corporation
Guanine hydrochloride	Nutritional Biochemicals Corporation
Xanthine	Nutritional Biochemicals Corporation
Pyrimidine base	
Uracil	Nutritional Biochemicals Corporation

Vitamins	
p-Aminobenzoic acid	The British Drug Houses Ltd., London
B - 12	Nutritional Biochemicals Corporation
Crystalline biotin	Nutritional Biochemicals Corporation
Folic acid (crystalline)	Nutritional Biochemicals Corporation
i- Inositol	Eastman Kodak Company
Nicotinic acid	Nutritional Biochemicals Corporation
Calcium pantothenate (dextrorotatory)	Eastman Kodak Company
Pyridoxine hydrochloride	Nutritional Biochemicals Corporation
Riboflavin	Eastman Kodak Company
Thiamine hydrochloride	Merck and Co. Inc.
Carbon	
Dextrose (d-glucose)	Fisher Scientific Company, U.S.A.
Ethyl alcohol (95 per cent)	Canadian Industrial Alcohols and Chemicals Limited, Montreal
Inorganic salts	
Ferrous sulphate heptahydrate	The British Drug Houses Ltd.
Magnesium sulphate heptahydrate	Fisher Scientific Company
Manganese sulphate tetrahydrate	The British Drug Houses Ltd.
Potassium dihydrogen phosphate	Fisher Scientific Company
Potassium hydrogen phosphate	Fisher Scientific Company
Sodium chloride	Fisher Scientific Company

Miscellaneous substances

Acetic acid (99.5 per cent glacial)	Nichols Chemical Company Limited, Montreal
Bacto-Agar	Difco Laboratories Incorporated
Hydrochloric acid, C.P. (35 to 37 per cent)	General Chemical Company, New York, U.S.A.
Hydrochloric acid (normal)	Anachemia Chemicals Limited, Montreal
Sodium hydroxide (normal)	Anachemia Chemicals Limited

b. Culture Medium

Cultures were maintained in test tubes on 5 ml. quantity slopes of yeast extract glucose agar containing 2 per cent calcium carbonate. The medium was prepared as described on page 43. Subcultures were made at monthly intervals, incubated at room temperature for two days and stored in a refrigerator.

Before preparing the inocula each organism was grown on the described medium.

c. Media for Studying Physical Factors

A basic yeast extract medium was used and was sometimes adapted, as will be indicated, to suit the study of the effect of particular physical factors on acetic acid production by Acetobacter.

The basic medium was made by dissolving yeast extract (Difco) with tap water in the proportion 1 gm.: 97 ml., and

steaming the mixture for fifteen minutes. The solution was adjusted to pH 6.0 with normal hydrochloric acid, and was distributed in 97 ml. quantities in 250 ml. Erlenmeyer flasks in preparation of "test and control flasks" and in larger amounts in larger flasks in preparation of replacement media. All flasks of media were autoclaved for fifteen minutes at 15 lb. pressure. To complete the preparation of "test flasks" and corresponding replacement flasks sufficient ethyl alcohol was added in order that the latter-named substance should occupy 3 per cent of the final volume.

d. Media for Studying Chemical Factors

Generalization

An attempt was made to obtain acetic acid through fermentation in various synthetic media by Acetobacter species. The basic synthetic medium consisted of groups of inorganic salts, amino acids and ethyl alcohol. Groups of vitamins and pyrimidine and purine bases were added for particular experiments.

Substances and Quantities Used

The synthetic media used in the previous section were not conducive to acetic acid production. Since very little former work on chemical factors affecting acetic acid

production could be used as a guide, work on substances affecting the growth of Acetobacter was studied. After considering the substances used, their concentration and their effect on Acetobacter growth (pages 12, 13, 14 and 15), a synthetic medium was prepared using the chemicals found necessary or of interest, in concentrations most commonly and successfully used by previous investigators. In instances where diverse concentrations were used by different authors, a moderate concentration was used. The concentration of Bacto-Vitamin Free Casamino Acids was the same as the concentration of yeast extract used in the physical factor studies. The following concentrations of compounds per 100 ml. medium were used:

Inorganic salts	
Ferrous sulphate heptahydrate	0.001 gm.
Magnesium sulphate heptahydrate	0.020 gm.
Manganese sulphate tetrahydrate	0.001 gm.
Potassium hydrogen phosphate	0.050 gm.
Potassium dihydrogen phosphate	0.050 gm.
Sodium chloride	0.001 gm.
Amino acids	
Bacto-Vitamin Free Casamino Acids	1.0 gm.
l(+) Cysteine hydrochloride	25.0 mg.
DL Norleucine	50.0 mg.
l- Tryptophane	25.0 mg.
Vitamins	
p-Aminobenzoic acid	20.0 µg.
B - 12	0.1 µg.

Vitamins continued

Biotin	0.5 µg.
Folic acid	10.0 µg.
i - Inositol	2.5 mg.
Nicotinic acid	100.0 µg.
Calcium d-pantothenate	100.0 µg.
Pyridoxine hydrochloride	50.0 µg.
Riboflavin	12.0 µg.
Thiamine hydrochloride	100.0 µg.

Pyrimidine base

Uracil	2.0 mg.

Purine bases

Adenine sulphate	2.0 mg.
Guanine hydrochloride	2.0 mg.
Xanthine	1.2 mg.

Solutions

Amino acids were added directly to the medium being made. Solutions of groups of salts were made to facilitate their use, and solutions of individual vitamins and pyrimidine and purine bases to measure accurately small quantities and in some instances to dissolve them. Most substances that are soluble in amounts used, as stated by the "Handbook of Chemistry and Physics" (13), were dissolved in distilled water. Vitamin solutions contained in ampoules were diluted

with distilled water according to the instructions. Other substances were dissolved according to indicated established procedures. Folic acid was diluted with distilled water because a method of solution at the required concentration was unobtainable. The quantity of solution required to make 1000 ml. medium is given for all solutions as any medium was usually prepared in 1000 ml. quantities or more. Solutions were stored in a refrigerator and renewed at monthly intervals.

Inorganic salts

Solution A:- Snell and Strong (39)

Twenty-five grams of potassium hydrogen phosphate and 25.0 gm. of potassium dihydrogen phosphate are dissolved in 250 ml. of distilled water.

5 ml. solution contains 0.5 gm. potassium hydrogen phosphate / 0.5 gm. potassium dihydrogen phosphate

Solution B:- Snell and Strong (39)

One-half gram of ferrous sulphate heptahydrate, 10.0 gm. of magnesium sulphate heptahydrate, 0.5 gm. of manganese sulphate tetrahydrate and 0.5 gm. of sodium chloride are dissolved in 250 ml. of distilled water.

5 ml. solution contains 0.01 gm. ferrous sulphate heptahydrate / 0.20 gm. magnesium sulphate heptahydrate / 0.01 gm. manganese sulphate tetrahydrate / 0.01 gm. sodium chloride

Vitamins

p-Aminobenzoic acid:- Barton-Wright (4)

Four milligrams of p-aminobenzoic acid is dissolved in 100 ml. of distilled water containing 1 ml. of glacial acetic acid.

5 ml. solution contains 200.0 µg.

B - 12: - The 1 ml. liquid content of an ampoule containing 15.0 µg. is diluted with 74 ml. of distilled water.

5 ml. solution contains 1.0 µg.

Biotin:- The 1 ml. liquid content of an ampoule containing 25.0 µg. is diluted with 24 ml. of distilled water.

5 ml. solution contains 5.0 µg.

Folic acid:- Two milligrams of folic acid was dissolved in 100 ml. of distilled water.

5 ml. solution contains 100.0 µg.

i-Inositol:- Five hundred milligrams of i-inositol is dissolved in 100 ml. of distilled water.

5 ml. solution contains 25.0 mg.

Nicotinic acid:- Barton-Wright (4)

Twenty milligrams of nicotinic acid is dissolved in 100 ml. of distilled water.

5 ml. solution contains 1000.0 µg.

Calcium d-pantothenate:- Barton-Wright (4)

Twenty milligrams of calcium d-pantothenate is dissolved in 100 ml. of distilled water.

5 ml. solution contains 1000.0 µg.

Pyridoxine hydrochloride:- Ten milligrams of pyridoxine hydrochloride is dissolved in 100 ml. of distilled water.

5 ml. solution contains 500.0 µg.

Riboflavin:- Barton-Wright (4)

Six milligrams of riboflavin is mixed with a little distilled water and 0.25 ml. of glacial acetic acid is added. The solution is diluted with distilled water to occupy 250 ml.

5 ml. solution contains 120.0 µg.

Thiamine hydrochloride:- Twenty milligrams of thiamine hydrochloride is dissolved in 100 ml. of distilled water.

5 ml. solution contains 1000.0 µg.

Pyrimidine base

Uracil:- György (11)

One hundred milligrams of uracil is dissolved with the aid of heat in 5 ml. of 20 per cent hydrochloric acid. The solution is diluted with distilled water to occupy 50 ml.

10 ml. solution contains 20.0 mg.

Purine bases

Adenine sulphate:- György (11)

One hundred milligrams of adenine sulphate is dissolved with the aid of heat in 5 ml. of 20 per cent hydrochloric acid. The solution is diluted with distilled water to occupy 50 ml.

10 ml. solution contains 20.0 mg.

Guanine hydrochloride:- György (11)

One hundred milligrams of guanine hydrochloride is dissolved with the aid of heat in 5 ml. of 20 per cent hydrochloric acid. The solution is diluted with distilled water to occupy 50 ml.

10 ml. solution contains 20.0 mg.

Xanthine:- Two hundred and forty milligrams of xanthine is dissolved in 100 ml. of distilled water with the aid of heat.

5 ml. solution contains 12.0 mg.

Media

The basic medium to study the chemical factors affecting acetic acid production from ethyl alcohol was prepared in the same manner as the medium to study the physical factors. Measured quantities of inorganic salts and amino acids replaced yeast extract. Vitamins and pyrimidine and purine bases were added to determine whether they would enhance acetic acid production.

A definite procedure was followed in adding substances so that the required weight of compounds was contained in the final medium. The number of milliliters of solutions containing appropriate weights of inorganic salts, inorganic salts and vitamins, or inorganic salts - vitamins and pyrimidine and purine bases were added to appropriate measured

quantities of amino acids. The total mixture was diluted with sufficient distilled water so that after the addition of ethyl alcohol the concentrations of the individual substances were correct.

4. Adaptation of the Media and Procedure to Suit the Study of the Effect of Varying Individual Factors on Acetic Acid Production

The relation of variations of the following physical factors to acetic acid production was studied:

The incubation temperature of the inoculated media.

The surface area of the inoculated media.

The sampling-time intervals of the fermenting media.

The initial ethyl alcohol content of the media.

The initial pH of the media.

The size of inocula in the media.

Mixed inocula in the media.

The relation of the following synthetic media to acetic acid production was studied:

Medium I:- Inorganic salts + amino acids

Medium II:- Inorganic salts + amino acids + vitamins

Medium III:- Inorganic salts + amino acids + vitamins + pyrimidine and purine bases &

Physical and chemical conditions under which variations of the first physical factor studied were performed, were

& - For individual substances see pages 117-118.

influenced by the work done in the previous section, and by the literature on acetic acid fermentation and growth of Acetobacter. All conditions were constant in the group of experiments except the factor being studied. The factor was varied within probable limits of obtaining acetic acid during fermentation. As was stated previously each organism was tested with every variation of the factor. The variation of the factor giving a maximum yield of acetic acid for individual organisms was integrated into succeeding groups of experiments as a fixed variation of the factor. All subsequent variations of factors giving a maximum yield of acetic acid by each organism were treated similarly. In some instances the variation of the factor giving an optimum yield of acetic acid seemed more important. It was therefore used. Sometimes, due to the results obtained, one variation of the factor was used for all organisms in all succeeding tests. All deviations will be clearly indicated. With the described procedure it was hoped that the final group of experiments on fermentation, the variation in chemical factors, would be performed under the best physical conditions for each organism.

Lists of fixed and changing variations of physical and chemical factors used during a given group of experiments are included with the discussion of the adaptation of the media and procedure to suit the study of the effect of individual factors on acetic acid production. When the maximum

or optimum yield of acetic acid per group of organisms is indicated, the variation used during the experiments is included in the list. When the variation giving the maximum yield per organism is indicated, the variation used may be obtained by referring to the stated Appendix Tables. Pertinent facts, related to the methods used, are explained. Lists are arranged relevant to the order of the study of the factors, and are more fully explained in the part on incubation temperature.

In any group of experiments after the first, the results of one variation of the factor studied were represented in the previous group, namely the variation which gave a maximum or optimum amount of acid by each organism with the fixed variation of the factor that was later studied.

a. The Effect of Varying Physical Factors on Acid Production

i. The Incubation Temperature of the Inoculated Media

The fixed and changing variations of physical and chemical factors used during fermentation in the group of experiments were as follows:

Initial yeast extract content (Difco)	1 per cent
VARIABLE - Incubation temperature	12 to 24°, 25°, 30° and 35°C.
Surface area	43.9 sq. cm. (250 flask)
Sampling-time intervals	3, 5, 8-day

Number of flasks per organism type	2 test, 1 control
Initial ethyl alcohol content	3 per cent
Initial pH	6.0
Size of inoculum	1 ml. from 1 slope suspension
Inoculum	single type of organism

The medium was prepared as described on pages 115-116. One per cent yeast extract adjusted to pH 6.0 was distributed in 97 ml. quantities in 250 ml. Erlenmeyer flasks. Three per cent of ethyl alcohol was added in preparation of test flasks. Two test and one control flask were prepared for inoculation with each type of bacterium for every variation of the factor being studied, temperature. The inoculum consisted of 1 ml. of a suspension of an organism per flask, prepared as described on page 111. Samples were removed at three, five and eight-day intervals and tested for acid content.

The incubation temperatures of the fermenting media were 12-24°, 25°, 30° and 35° C. Facilities for controlling accurately the lower temperature were not available.

ii. The Surface Area of the Inoculated Media

The fixed and changing variations of physical and chemical factors used during fermentation in the group of experiments were as follows:

Initial yeast extract content (Difco)	1 per cent
Incubation temperature	maximum per organism (Appendix Table VII)
VARIABLE - Surface area	43.9 sq.cm.,250 flask 76.6 sq.cm.,500 flask
Sampling-time intervals	3, 5, 8-day
Number of flasks per organism type	2 test, 1 control
Initial ethyl alcohol content	3 per cent
Initial pH	6.0
Size of inoculum	1 ml. from 1 slope suspension
Inoculum	single type of organism

Fermenting media were contained in 250 ml. and 500 ml. Erlenmeyer flasks to vary the surface area. The surface area at the meniscus of the media in 250 ml. flasks was approximately 43.9 sq. cm., and the surface area in 500 ml. flasks was approximately 76.6 sq. cm. Individual inoculated media were incubated at maximum acetic acid-producing temperatures for each organism.

iii. The Sampling-Time Intervals of the Fermenting Media

The fixed and changing variations of physical and chemical factors used during fermentation in the group of experiments were as follows:

Initial yeast extract content (Difco)	1 per cent
Incubation temperature	maximum per organism (Appendix Table VII)
Surface area	optimum per group 43.9 sq.cm.,250 flask
VARIABLE - Sampling-time intervals	3, 5, 8-day 2, 4, 6, 8, 10-day
Number of flasks per organism type	2 test, 1 control 3 test, 1 control
Initial ethyl alcohol content	3 per cent
Initial pH	6.0
Size of inoculum	1 ml. from 1 slope suspension
Inoculum	single type of organism

Fermentation samples were removed at three, five and eight-day and at two, four, six, eight, and ten-day intervals to test their acidity. Media were placed in 250 ml. Erlenmeyer flasks since any extra amount of air obtained by the organisms did not prove beneficial in studying acid production. A satisfactory maximum average amount of acid was always formed in 250 ml. flasks, but was sometimes formed at a slower rate during the beginning of the fermenting period. The smaller flasks also required less space. In setting up the experiment for taking two, four, six, eight and ten-day samples three test and one control flasks were prepared, because identical flasks of inoculated media often produced greatly differing quantities of acetic acid.

iv. The Initial Ethyl Alcohol Content of the Media

The fixed and changing variations of physical and chemical factors used during fermentation in the group of experiments were as follows:

Initial yeast extract content (Difco)	1 per cent
Incubation temperature	maximum per organism (Appendix Table X)
Surface area	optimum per group 43.9 sq.cm.,250 flask
Sampling-time intervals	optimum per group 2, 4, 6, 8, 10-day
Number of flasks per organism type	3 test, 1 control
VARIABLE - Initial ethyl alcohol content	3, 6 and 9 per cent
Initial pH	6.0
Size of inoculum	1 ml. from 1 slope suspension
Inoculum	single type of organism

A study of the effect of 3, 6, 9 and 12 per cent ethyl alcohol in the initial fermenting medium was intended. However, since very little acid was produced in inoculated 9 per cent nutrient media, no further work was done. In order to obtain the required final concentration of ethyl alcohol in the nutrient media the yeast extract, water and ethyl alcohol were combined in the following proportions:

1 gm.: 94 ml.: 6 ml. in the 6 per cent alcohol containing media

1 gm.: 91 ml.: 9 ml. in the 9 per cent alcohol containing media

Readings were taken at two, four, six, eight and ten-day intervals. A more accurate determination of the highest amount of acid produced was determined, since sampling-time intervals were not spaced too widely apart.

v. The Initial pH of the Media

The fixed and changing variations of physical and chemical factors used during fermentation in the group of experiments were as follows:

Initial yeast extract content (Difco)	1 per cent
Incubation temperature	maximum per organism (Appendix Table XV)
Surface area	optimum per group 43.9 sq.cm.,250 flask
Sampling-time intervals	optimum per group 2, 4, 6, 8, 10-day
Number of flasks per organism type	3 test, 1 control
Initial ethyl alcohol content	maximum per organism (Appendix Table XV)
VARIABLE - Initial pH	4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0
Size of inoculum	1 ml. from 1 slope suspension
Inoculum	single type of organism

When preparing the media the pH was adjusted to 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0 for successive tests. pH adjustments were made with normal hydrochloric acid or sodium hydroxide. Media containing maximum acid-producing quantities

of ethyl alcohol were prepared for each organism.

vi. The Size of Inocula in the Media

The fixed and changing variations of physical and chemical factors used during fermentation in the group of experiments were as follows:

Initial yeast extract content (Difco, Nutritional Biochemicals Corporation)	1 per cent
Incubation temperature	maximum per organism (Appendix Table XXIII)
Surface area	optimum per group 43.9 sq.cm.,250 flask
Sampling-time intervals	optimum per group 2, 4, 6, 8, 10-day
Number of flasks per organism type	3 test, 1 control
Initial ethyl alcohol content	maximum per organism (Appendix Table XXIII)
Initial pH	maximum per organism (Appendix Table XXIII)
VARIABLE - Size of inoculum	1 ml. from 1, 3 and 5 slope suspensions
Inoculum	single type of organism

Standard, three times and five times standard sized inocula were used. To prepare an inoculum three times the standard size three inoculated slopes, instead of one, were washed and the organisms were suspended in the same amount of water. The organisms were washed as described on page 111, and each appropriate test flask and control was inoculated with 1 ml. of the final suspension. An inoculum five times the standard size

was prepared in the same manner with five slopes. When testing the effect of three and five-slope inocula on acetic acid production, yeast extract from the Nutritional Biochemicals Corporation was substituted for that from Difco Laboratories in the slope and fermentation media. Media adjusted to maximum acid-producing pH's 5.0, 5.5 or 6.0 were used for nine types of inocula; media adjusted to second best acid-producing pH's 5.0, 5.5 or 6.0 were used for the remaining four types of inocula, since maximum and second best yields of acid were practically the same.

vii. Mixed Inocula in the Media

The fixed and changing variations of physical and chemical factors used during fermentation in the first type of experiment were as follows:

Initial yeast extract content (Difco, Nutritional Biochemicals Corporation)	1 per cent
Incubation temperature	maximum per organism (Appendix Table XXVII)
Surface area	optimum per group 43.9 sq.cm.,250 flask
Sampling-time intervals	optimum per group 2, 4, 6, 8, 10-day
Number of flasks per organism type	3 test, 1 control
Initial ethyl alcohol content	maximum per organism (Appendix Table XXVII)
Initial pH	maximum per organism (Appendix Table XXVII)

Size of inoculum	1 ml. from 2 slope suspension (i.e., 2 organisms)
VARIABLE - Inoculum	mixed - each organism type alone, each organism type + A. Aceti 1

Each organism was tested alone and with an equal amount of Acetobacter aceti 1, an organism that produced a good quantity of acid in medium containing 3 per cent ethyl alcohol but not 6 per cent, to determine the effect the mixtures had on acetic acid production. A one slope culture of an individual organism plus a one slope culture of Acetobacter aceti 1 was washed, and the organisms were suspended in the amount of water used for all other tests. The mixture of organisms was washed as described on page 111, and 1 ml. of the final suspension was inoculated into each appropriate test flask and control. To test single slopes 1 ml. of one slope suspensions was used.

The fixed and changing variations of physical and chemical factors used during fermentation in the second type of experiment were as follows:

Initial yeast extract content (Nutritional Biochemicals Corporation)	1 per cent
Incubation temperature	25° C.ç
Surface area	43.9 sq.cm.,250 flask
Sampling-time intervals	optimum per group 2, 4, 6, 8, 10-day
Number of flasks per organism type	3 test, 1 control
Initial ethyl alcohol content	6 per centç

ç - These conditions proved most favourable for acetic acid production with most organisms.

Initial pH	5.5[9]
Size of inoculum	1 ml. from suspension of all organisms.
VARIABLE - Inoculum	mixed - all organisms

Inocula in individual flasks were represented by each type of organism. Appropriate water suspensions of each type of organism were made. One milliliter of each suspension was placed in a test tube and the mixture was washed as described on page 111. When the final supernatent wash was poured off, the organisms were suspended in 13 ml. water. Appropriate test and control flasks were inoculated with 1 ml. of the final suspension. The final inoculum, therefore, consisted of the proper quantity of inoculum, that was represented by one-thirteenth of each of the thirteen components. The rate of acid production was compared with the rate using individual inocula.

b. The Effect of Varying Chemical Factors on Acid Production

The fixed and changing variations of physical and chemical factors used during fermentation in the group of experiments were as follows:

VARIABLE - Initial media	Media I, II, III
Incubation temperature	maximum per organism (Appendix Table XXXI)
Surface area	optimum per group 43.9 sq.cm.,250 flask

Sampling-time intervals	optimum per group 2, 4, 6, 8, 10-day
Number of flasks per organism type	3 test, 1 control
Initial ethyl alcohol content	maximum per organism (Appendix Table XXXI)
Initial pH	maximum per organism (Appendix Table XXXI)
Size of inoculum	1 ml. from 1 slope suspension
Inoculum	single type of organism

Medium I consisted of inorganic salts and amino acids. Medium II consisted of inorganic salts, amino acids and vitamins. Medium III consisted of inorganic salts, amino acids, vitamins and pyrimidine and purine bases. The media were made as described on pages 116-123. In making Medium III l(+) cysteine hydrochloride from Eastman Kodak Ltd. was used in place of that from Merck and Co., and l-trytophane from Nutritional Biochemicals Corporation in place of that from Difco Laboratories (see pages 113-115). Subcultures were made during Experiment 3 with yeast extract from the Difco Laboratories. Media containing 1 ml. from one-slope washed suspensions were used. In terms of maximum acid production all organisms fermented alcohol satisfactorily when standard sized inocula were used. Samples from the fermenting Medium III were titrated with the aid of the Fisher Titrimeter.

F. RESULTS

The results of all experiments are contained in Appendix Tables I to XXXIV (pp.244-278). The tables include the averages of the sample titrations of each type of inoculated "test flask"; the titration of samples from each inoculated "control flask"; and the average amount of acetic acid calculated from the average titration readings. The figures plotted to study the rate of acid production, on the following pages, were made from the tables. After determining the effect of the variation of each factor, a table was made with the maximum average production of acetic acid for each variation and organism.

During fermentation experiments, Acetobacter suboxydans, Acetobacter pasteurianum, and Acetobacter turbidans did not produce more than 0.15 gm. of acetic acid per 100 ml. of media. Therefore, they will not be discussed further.

1. Spasmodic Production of Acetic Acid by Acetobacter Species

After all experiments were completed, it was observed that great differences in acetic acid production sometimes existed in fermenting media under identical conditions, and that organisms varied in their ability to produce similar amounts of acetic acid. The observations were made from twelve tests with all organisms; the tests were the effect of ethyl alcohol concentration, pH, and the size of the inoculum. These tests were selected because three, instead of two, identical fermenting media were set up in a known

nutritious medium, and individual inocula were used. The titration readings of the three samples from identical fermenting flasks were arranged in order of magnitude. When the differences between the smallest and the second or the second and the third were not within the arbitrary limits of 0 to 8.99 ml. their incidence was recorded (Appendix Table I, pp.244-245). The method seemed practical because organisms so-arranged within 6.0 to 8.99 and 3.0 to 5.99 ml. limits never produced a titration difference between the smallest and the largest of over 9.0 ml.; organisms within 3.0 to 5.99 ml. limits seldom produced a titration difference between the smallest and the largest of over 6.0 ml.

Acetobacter gluconicum and Acetobacter melanogenum 1 were so spasmodic in acetic acid production that a good yield was only produced in one instance; during these tests titration differences between fermenting samples were as great as 34.5 and 21.0 ml. respectively.

Acetobacter rancens produced sufficient acetic acid during tests, but great titration differences. The titration difference between fermenting samples in one experiment during one-day readings was 41.5 ml., and in seven other experiments differences were 9.0 to 26.8 ml. Acetobacter oxydans 2 during one test produced a titration difference of 21.9 ml. and during three other experiments differences of 9.0 to 13.9 ml. Acetobacter kützingianum produced titration differences of 9.0 to 10.9 ml. during four experiments. Acetobacter oxydans 1 and Acetobacter acetosum produced titration differences of 9.0 to

11.99 ml. only on one occasion.

Next, taking the arbitrary limits of 6.0 to 8.99 ml. titration differences of this magnitude were observed for Acetobacter oxydans 1, Acetobacter acetosum and the remaining organisms.

Organisms, that had one or no titration difference within the 6.0 to 8.99 ml. limit, were tested in the 3.0 to 5.99 limit. After testing the latter limit all organisms were represented.

In most instances the titration differences were observed in the six, eight and ten-day samples. They were sometimes observed in the four-day samples.

In Table XXX the number of instances of titration differences within the given limits are indicated. Organisms are then classified in order of their spasmodic behaviour in producing acetic acid. Acetobacter gluconicum and Acetobacter melanogenum 1 are not included because they only produced a good acid yield on one occasion.

2. Arbitrary Point of Significance in Tests

The average titration represents the closest to the most probable answer if the tests were performed under the same conditions and the average taken again. Averages could differ, however, in amounts dependent on the organism being studied.

An arbitrary amount of 6 ml. of maximum average titration difference for acetic acid determination with two variations of the same factor using the same organism is considered

TABLE XXX

THE POSSIBILITY AND AMOUNT OF TITRATION DIFFERENCES IN SIMILAR FERMENTING FLASKS DURING TWELVE TESTS

Organism	Instances of titration difference between samples of similar fermenting media, ml								Degree of spasmodic acid production possible within test
	42	24-26.99	21-23.99	15-17.99	12-14.99	9-11.99	6-8.99	3-5.99	
A. rancens	1	3	1	2	1				++++
A. oxydans 2			1		2	1			+++
A. kützingianum						4			++
A. oxydans 1						1	3		+
A. acetosum						1	2		+
A. ascendens								3	0
A. aceti 1								3	0
A. aceti 2							1	5	0

significant. A 6 ml. titration represents 0.36 or 0.4 gm. of acetic acid per 100 ml. medium. It must be remembered that the more erratic the organism is in acid production, the less significant is the difference. Where more than two variations were studied the variation producing the highest maximum average yield is considered along with all other variations producing maximum yields that did not differ by as much as 0.4 gm. of acetic acid with the same organism. The variations producing lower yields are considered in the next category. The same difference is used as much as possible in the interpretation of the figures; figures are only plotted to the nearest 0.1 gm. of acetic acid. The only average differences that would be plotted erroneously as 0.4 gm. are 0.35 to 0.44. Changes less than 0.4 gm. in acid production are considered in daily average acetic acid yields on the same test for the same organism especially when certain characteristics in production appear to be dependent on the organism.

A maximum average production of 0.2 gm. of acetic acid per 100 ml. of media by any organism is arbitrarily considered significant.

In the discussion of the results of the effect of variations of factors on acetic acid production, the rate of acetic acid production and the maximum amount produced by one organism are not compared with those by any other organism. Each organism is discussed alone. Only the rate of acetic acid production and the maximum amount produced by an organism, with one variation of a factor, are compared with those by the same

organism with another variation of the factor. Organisms are only discussed together when variations of factors give the same trend in acetic acid production.

3. The Effect of Varying Physical Factors on the Rate and Amount of Acetic Acid Produced

i. The Incubation Temperature of the Inoculated Media

The results of the experiments are recorded in Figures 2 to 9 (pp.142-149), and Appendix Tables II to VI (pp.246-250).

During fermentation experiments Acetobacter gluconicum and Acetobacter melanogenum 1 produced insignificant amounts of acetic acid. In the results of the effect of variations of temperature and other physical factors on acetic acid production, Acetobacter gluconicum and Acetobacter melanogenum 1 are discussed separately because they were very spasmodic in acid production.

All other organisms produced a lag in acetic acid production, when fermenting flasks were incubated at 12° to 24° C.; Acetobacter acetosum, Acetobacter kützingianum, Acetobacter aceti 1, Acetobacter oxydans 1 and Acetobacter oxydans 2 produced a lag when fermenting flasks were incubated at 35° C. Acetobacter aceti 2, Acetobacter rancens, Acetobacter oxydans 1 and Acetobacter oxydans 2 produced a maximum amount of acid in less than eight days at 25° and 30° C. and the average yield then decreased. Therefore, the time at which a maximum yield of acetic acid is obtained with these organisms can be regulated by the incubation temperature of the fermenting media.

In terms of maximum acid production each organism oxidized

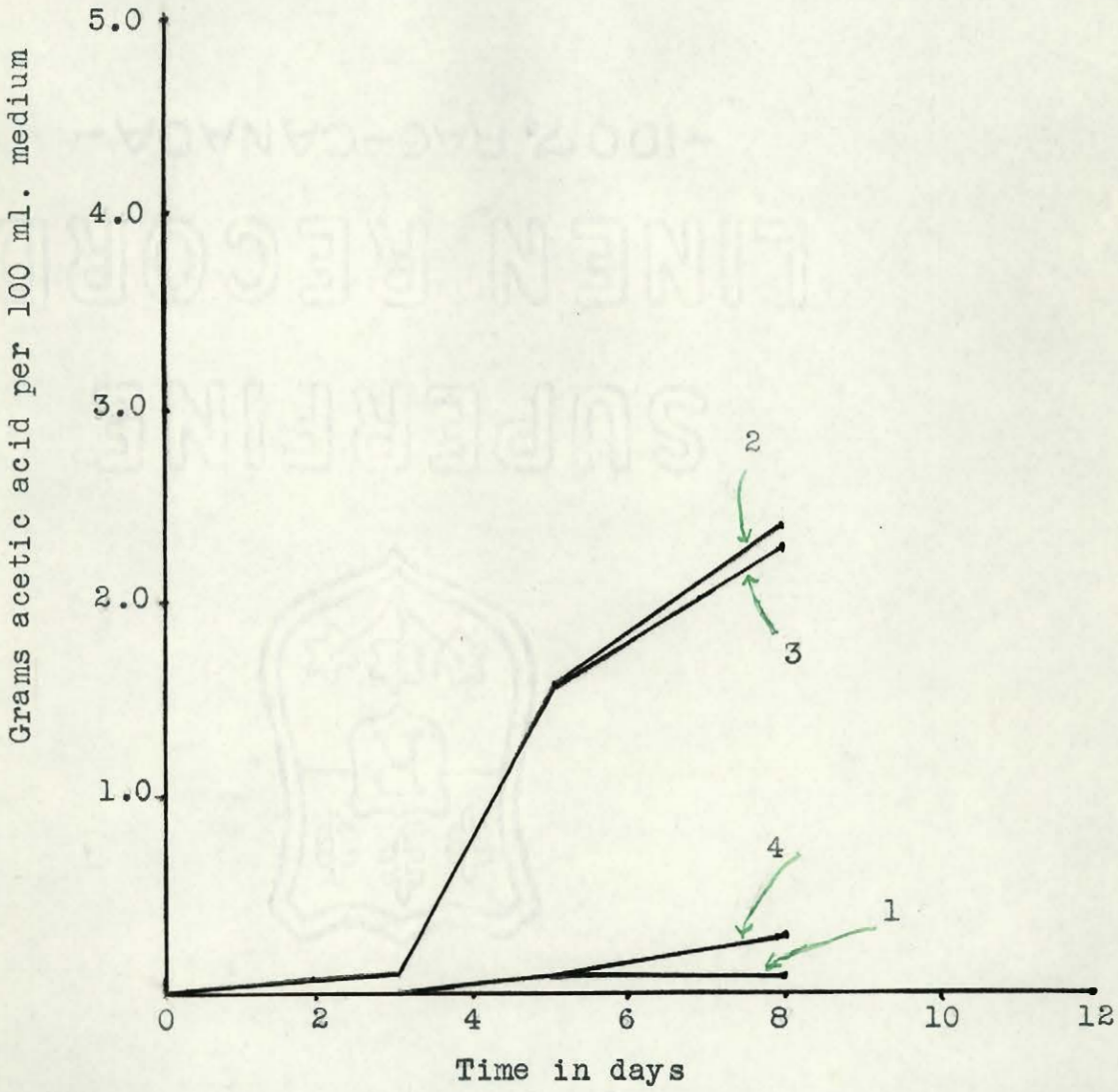


FIGURE 2 - THE EFFECT OF VARYING THE INCUBATION TEMPERATURE OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER ACETOSUM

Curve 1 - Incubation temperature 12 - 24° C
2 - Incubation temperature 25° C
3 - Incubation temperature 30° C
4 - Incubation temperature 35° C

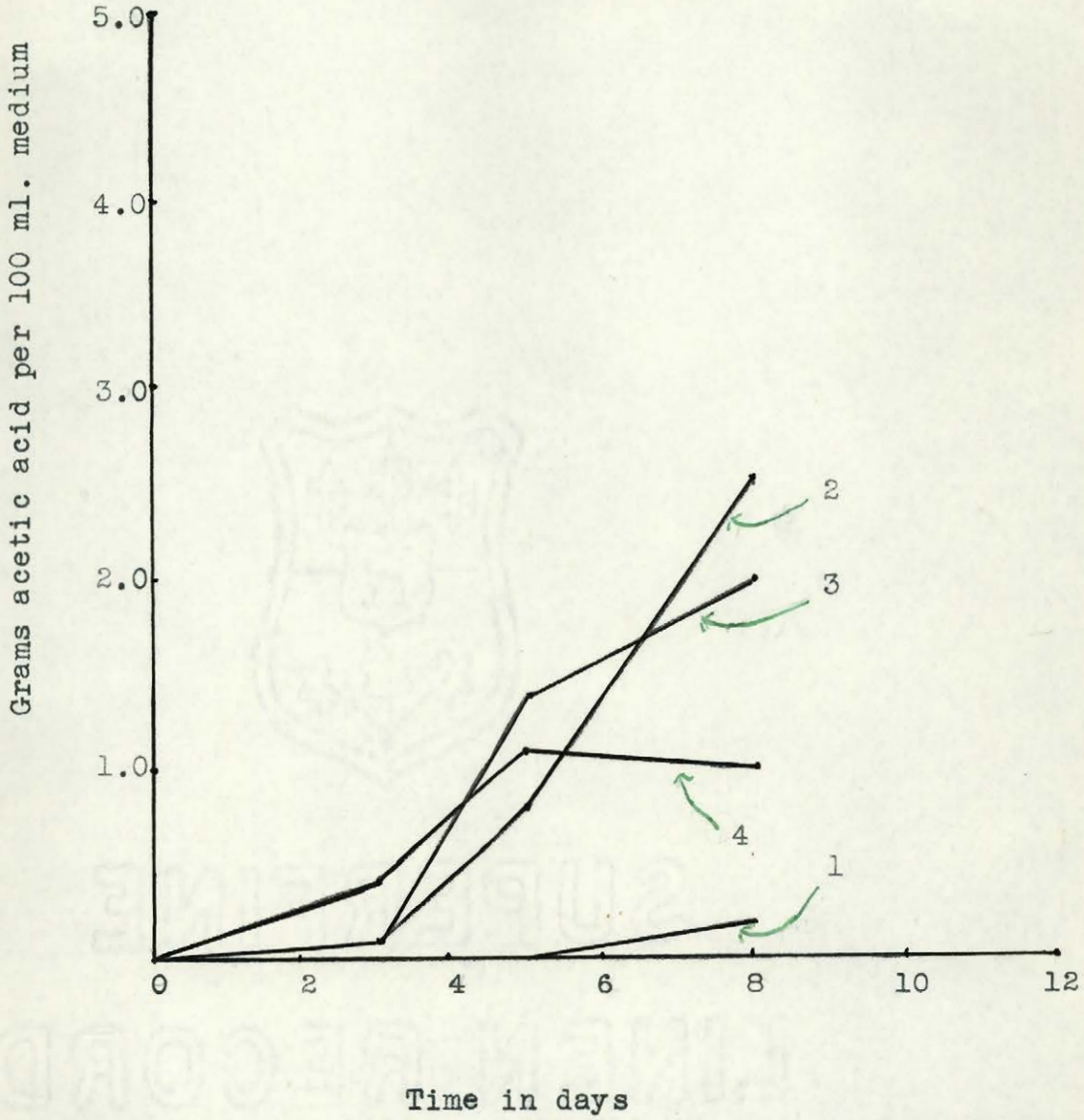


FIGURE 3 - THE EFFECT OF VARYING THE INCUBATION TEMPERATURE OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER ASCENDENS

Curve 1 - Incubation temperature 12 - 24° C
2 - Incubation temperature 25° C
3 - Incubation temperature 30° C
4 - Incubation temperature 35° C

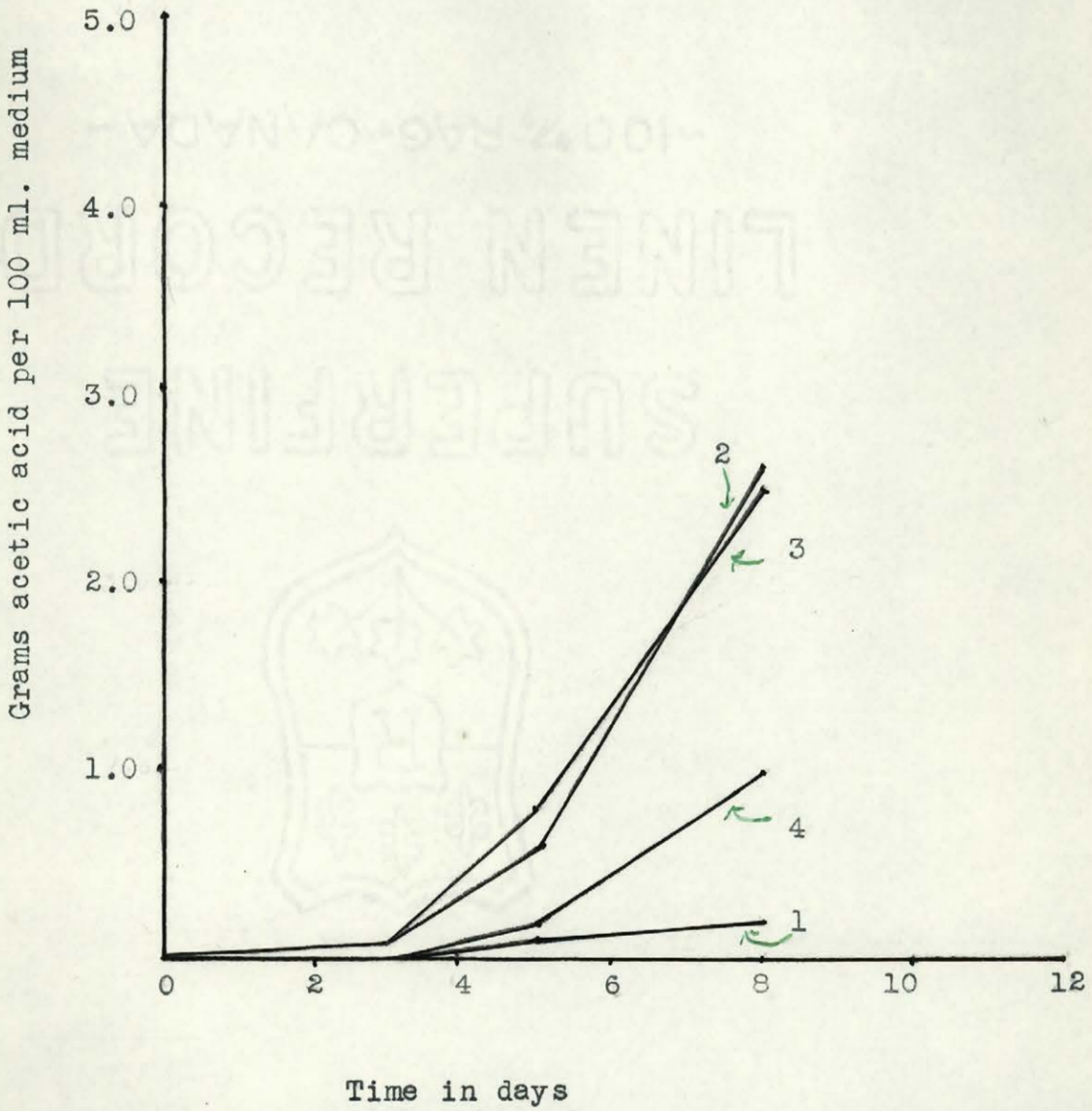


FIGURE 4 - THE EFFECT OF VARYING THE INCUBATION TEMPERATURE OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER KÜTZINGIANUM

Curve 1 - Incubation temperature 12 - 24° C
2 - Incubation temperature 25° C
3 - Incubation temperature 30° C
4 - Incubation temperature 35° C

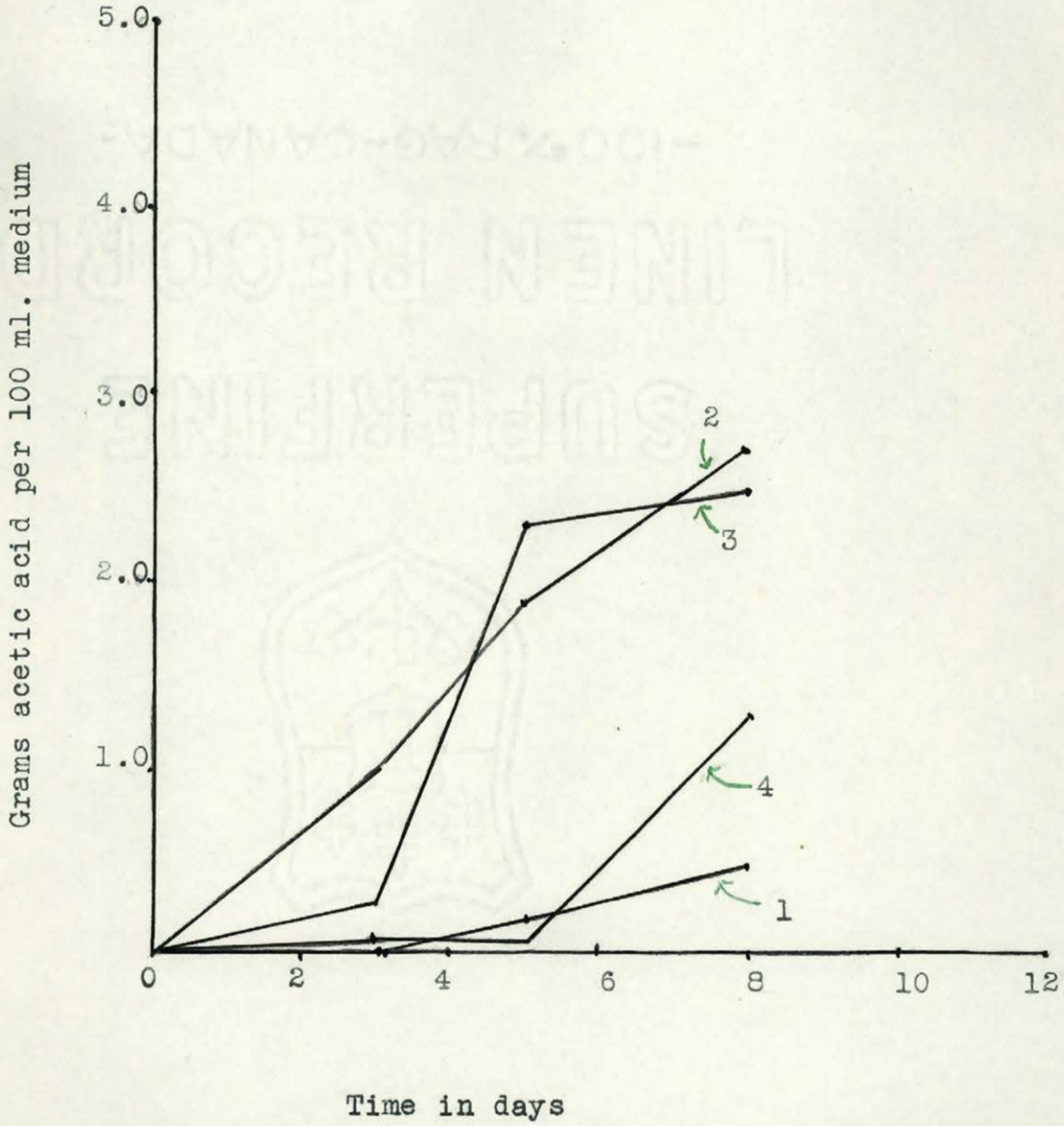


FIGURE 5 - THE EFFECT OF VARYING THE INCUBATION TEMPERATURE OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER ACETI 1

Curve 1 - Incubation temperature 12 - 24° C
2 - Incubation temperature 25° C
3 - Incubation temperature 30° C
4 - Incubation temperature 35° C

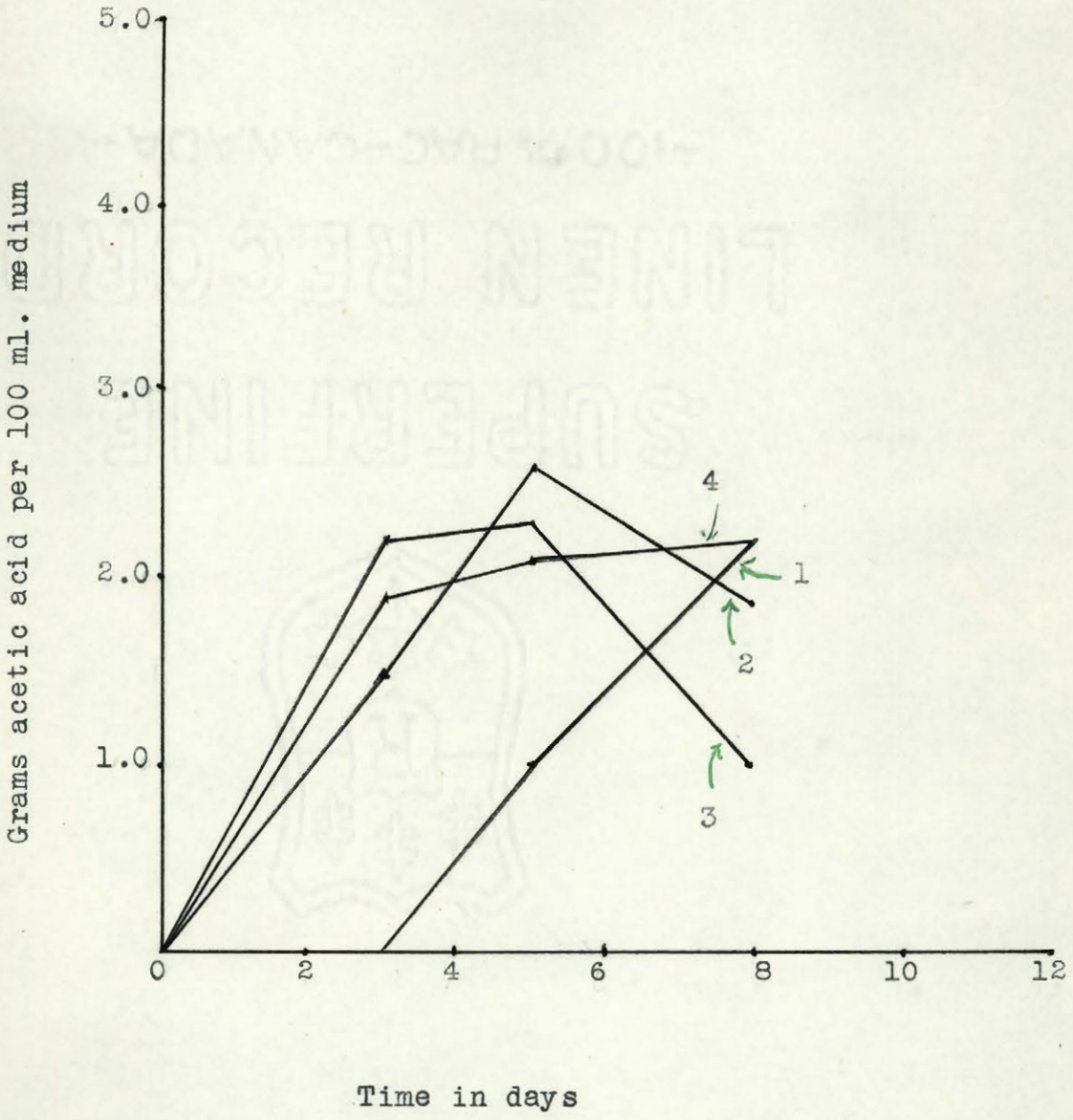


FIGURE 6 - THE EFFECT OF VARYING THE INCUBATION TEMPERATURE OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER ACETI 2

Curve 1 - Incubation temperature 12 - 24° C
2 - Incubation temperature 25° C
3 - Incubation temperature 30° C
4 - Incubation temperature 35° C

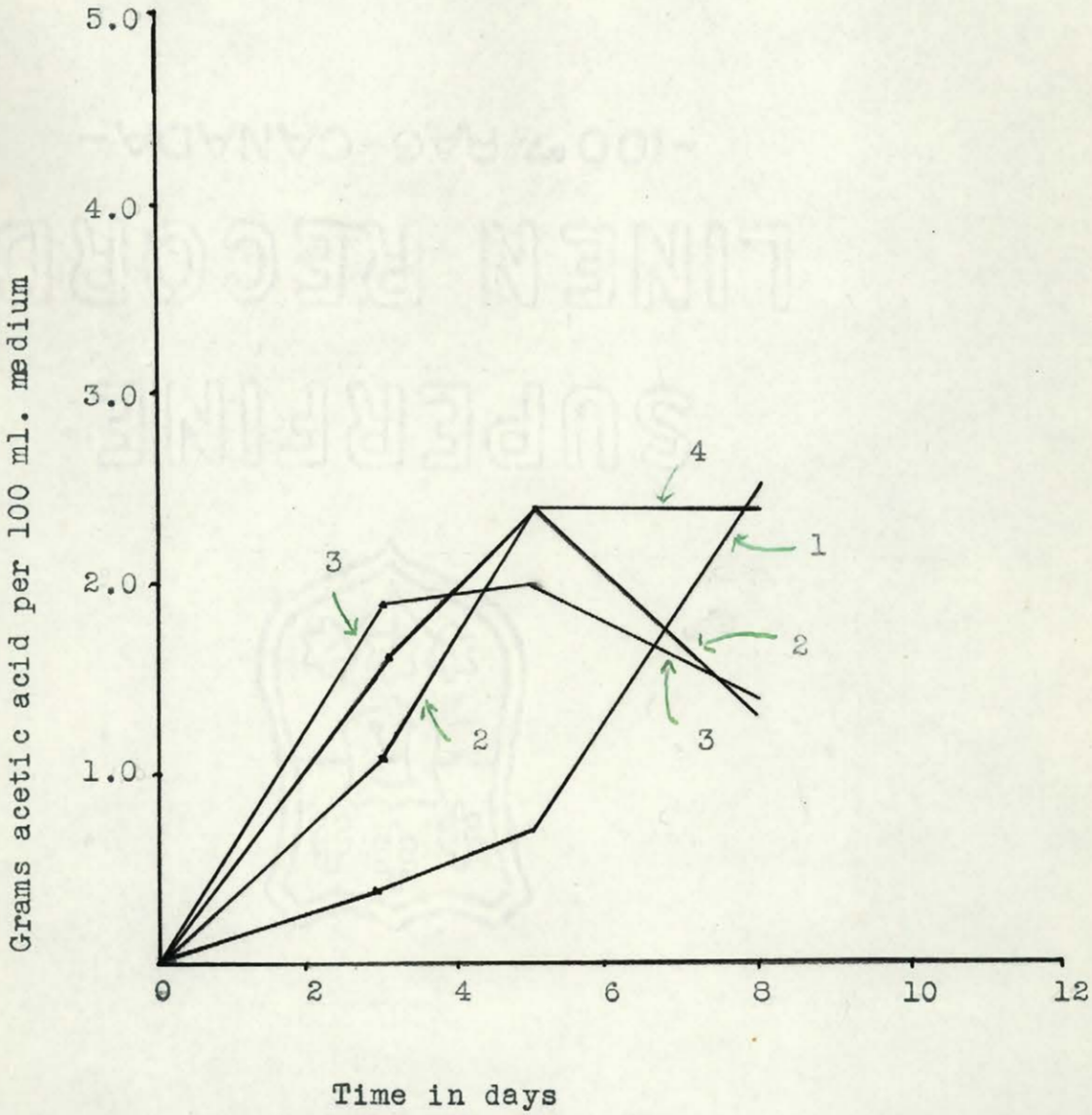


FIGURE 7 - THE EFFECT OF VARYING THE INCUBATION TEMPERATURE OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER RANCENS

Curve 1 - Incubation temperature 12 - 24° C
2 - Incubation temperature 25° C
3 - Incubation temperature 30° C
4 - Incubation temperature 35° C

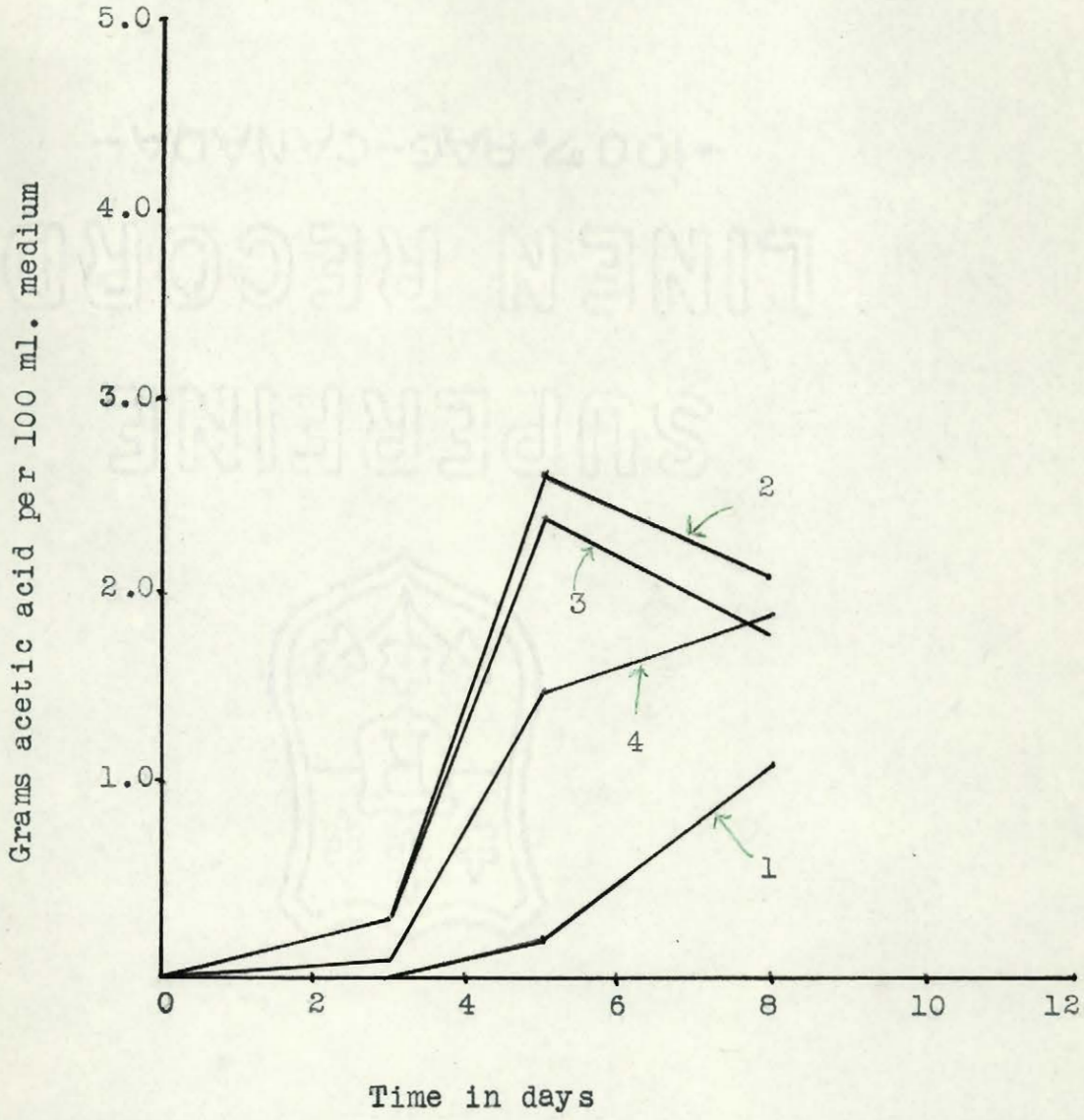


FIGURE 8 - THE EFFECT OF VARYING THE INCUBATION TEMPERATURE OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER OXYDANS 1

Curve 1 - Incubation temperature 12 - 24° C
2 - Incubation temperature 25° C
3 - Incubation temperature 30° C
4 - Incubation temperature 35° C

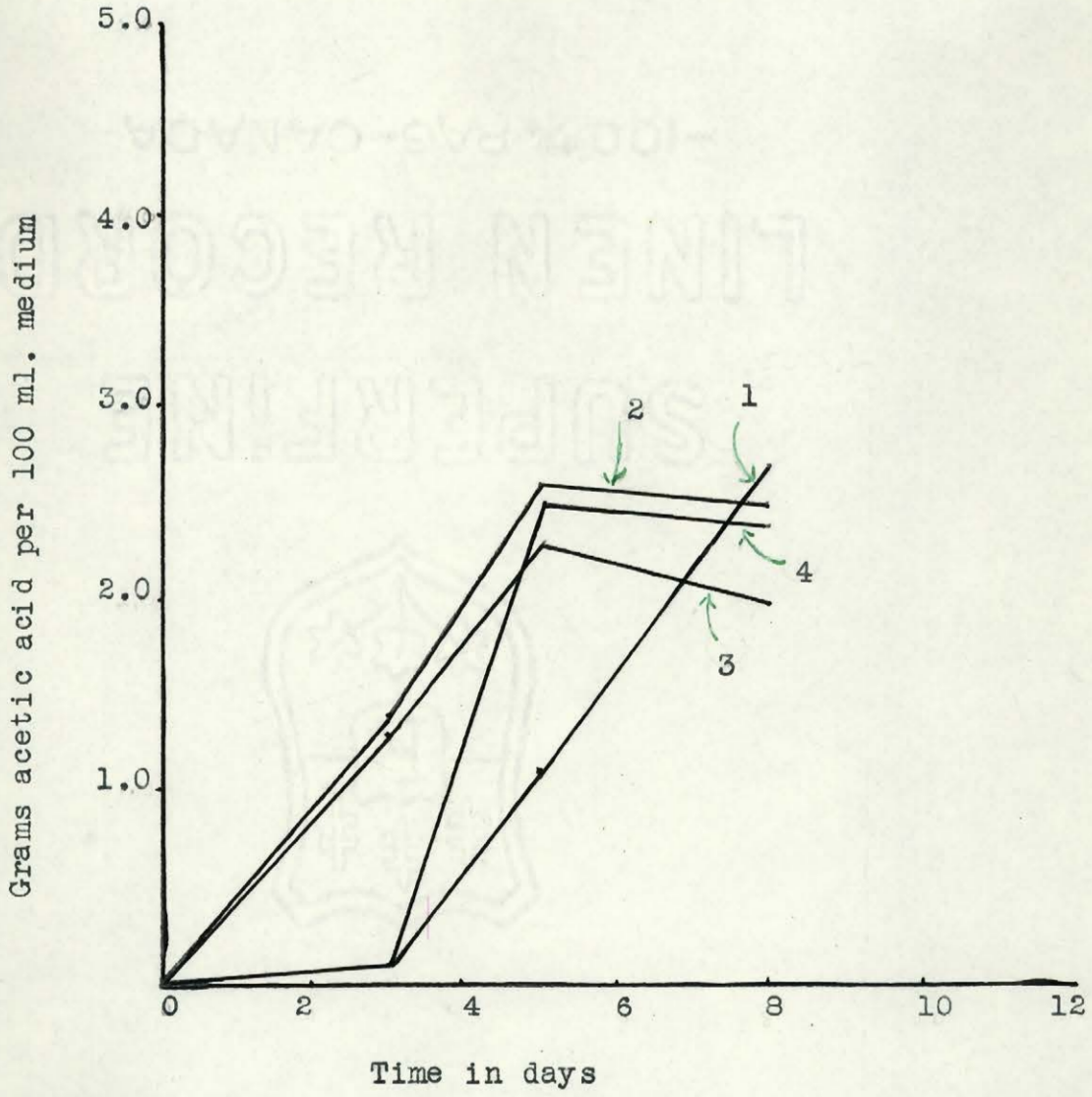


FIGURE 9 - THE EFFECT OF VARYING THE INCUBATION TEMPERATURE OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER OXYDANS 2

Curve 1 - Incubation temperature 12 - 24° C
2 - Incubation temperature 25° C
3 - Incubation temperature 30° C
4 - Incubation temperature 35° C

ethyl alcohol satisfactorily at 25° C. Acetobacter oxydans 2 produced insignificantly different maximum amounts of acetic acid at 12° to 24°, 25°, 30° and 35° C.; Acetobacter aceti 2 at 12° to 24°, 25° and 30° C.; Acetobacter rancens at 12° to 24°, 25° and 35° C.; and Acetobacter acetosum, Acetobacter kützingianum, Acetobacter aceti 1 and Acetobacter oxydans 1 at 25° and 30° C. Acetobacter ascendens produced a maximum amount of acid at 25° C.

ii. The Surface Area of the Inoculated Media.

The results of the experiments are recorded in Figures 10 to 17 (pp.151-158), and Appendix Tables VII to IX (pp.251-253).

During fermentation experiments Acetobacter gluconicum and Acetobacter melanogenum 1 produced insignificant amounts of acetic acid. The latter did produce a maximum yield of 0.24 gm. of acid per 100 ml. of medium, in 500 ml. Erlenmeyer flasks.

All other organisms tended to produce a greater lag in acetic acid production when fermentations took place in 250 ml. Erlenmeyer flasks, the flasks in which the surface area of the exposed liquid was the lesser. Acetobacter aceti 2 and Acetobacter oxydans 1 produced a maximum amount of acid in less than eight days, and the average yield then decreased with both types of flask; Acetobacter aceti 2 produced a maximum yield of acetic acid in a shorter interval of time when the surface area was the greater; Acetobacter acetosum, Acetobacter rancens, and Acetobacter oxydans 2 produced the same effect to a lesser extent usually in the flasks with the greater surface area.

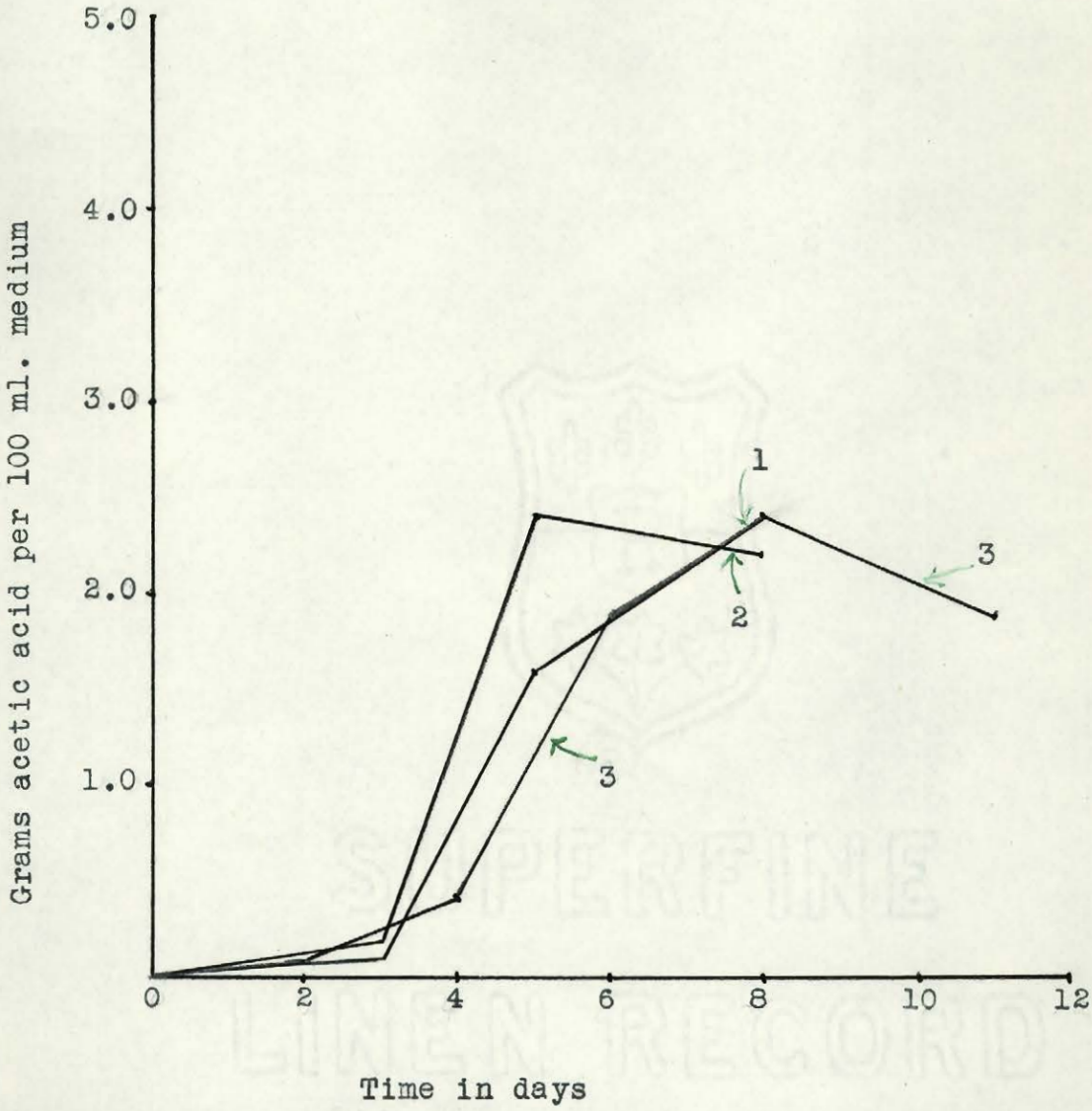


FIGURE 10 - I. THE EFFECT OF VARYING THE SURFACE AREA OF THE INOCULATED YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED. II. A COMPARISON OF EFFICIENCY IN STUDYING THE RATE OF ACID PRODUCTION BY USING DIFFERENT SAMPLING-TIME INTERVALS. ACETOBACTER ACETOSUM

Curve 1 - Surface area 43.9 sq.cm., sampling-time 3,5,8 days (250 ml. flask)
2 - Surface area 76.6 sq.cm., sampling-time 3,5,8 days (500 ml. flask)
3 - Surface area 43.9 sq.cm., sampling-time 2,4,6,8,10 days (250 ml. flask)

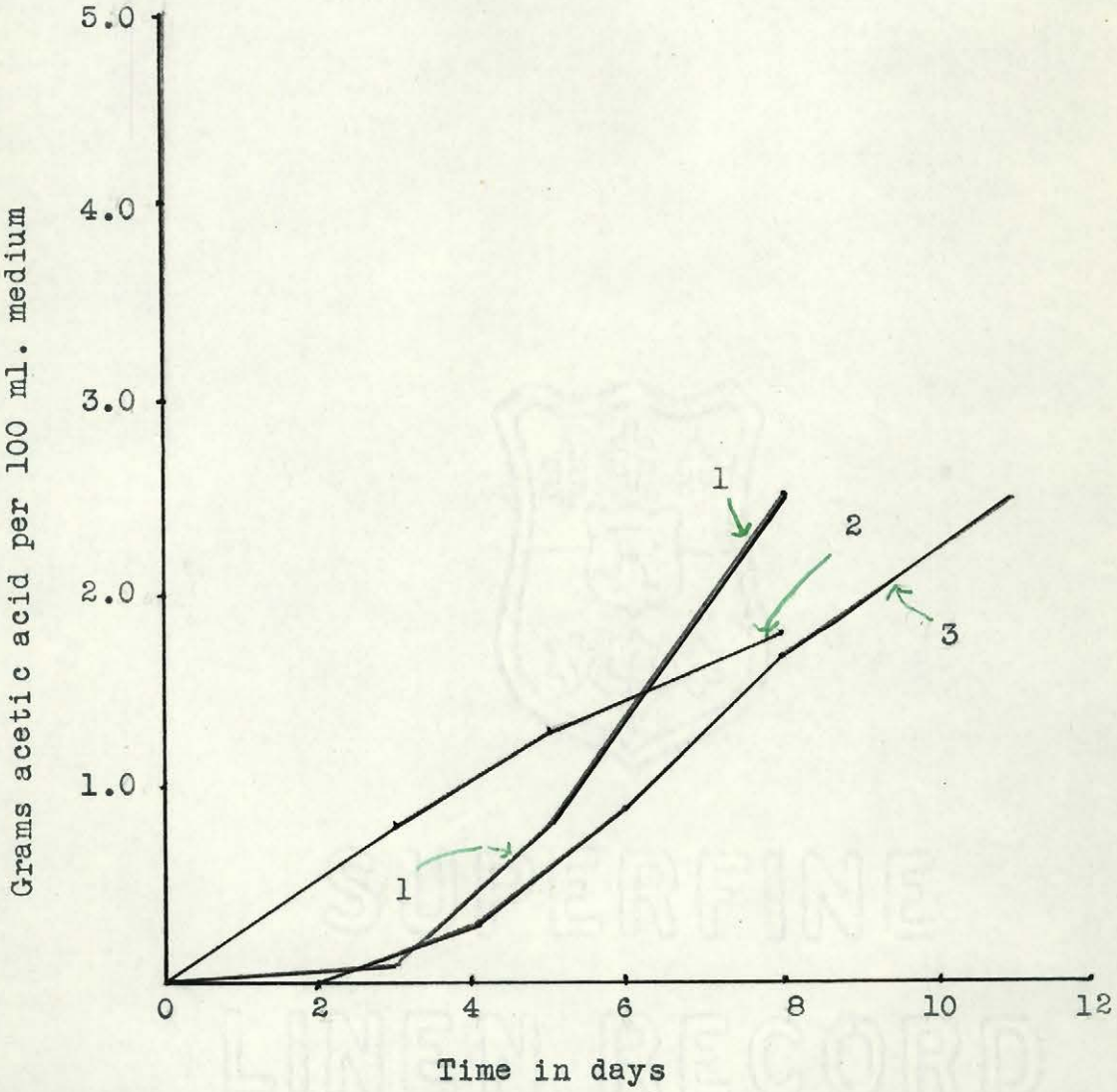


FIGURE 11 - I. THE EFFECT OF VARYING THE SURFACE AREA OF THE INOCULATED YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED. II. A COMPARISON OF EFFICIENCY IN STUDYING THE RATE OF ACID PRODUCTION BY USING DIFFERENT SAMPLING-TIME INTERVALS. ACETOBACTER ASCENDENS

Curve 1 - Surface area 43.9 sq. cm., sampling-time 3,5,8 days (250 ml. flask)
2 - Surface area 76.6 sq. cm., sampling-time 3,5,8 days (500 ml. flask)
3 - Surface area 43.9 sq. cm., sampling-time 2,4,6,8,10 days (250 ml. flask)

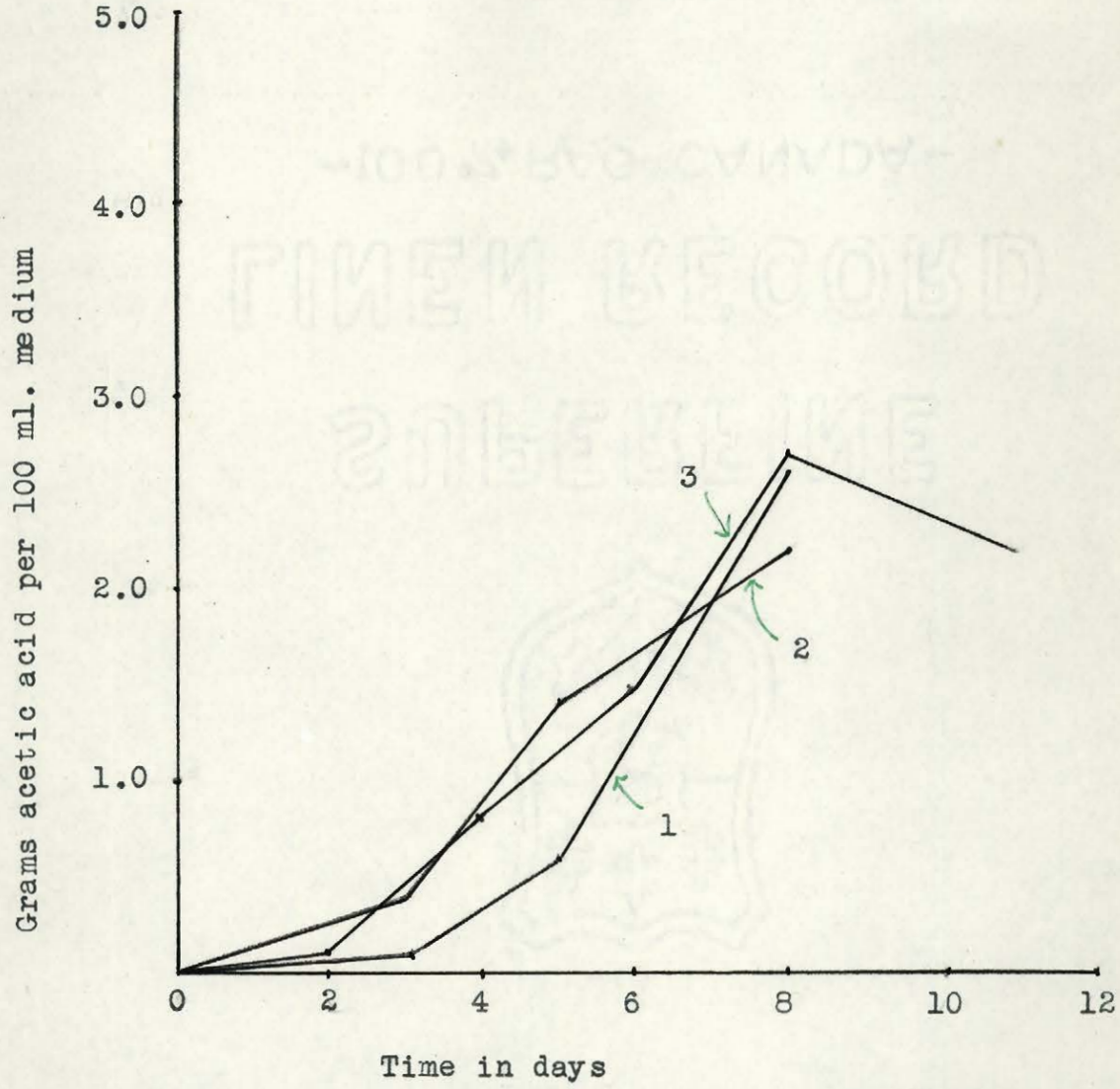


FIGURE 12 - I. THE EFFECT OF VARYING THE SURFACE AREA OF THE INOCULATED YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED. II. A COMPARISON OF EFFICIENCY IN STUDYING THE RATE OF ACID PRODUCTION BY USING DIFFERENT SAMPLING-TIME INTERVALS. ACETOBACTER KÜTZINGIANUM

Curve 1 - Surface area 43.9 sq.cm., sampling-time 3,5,8 days (250 ml. flask)
2 - Surface area 76.6 sq.cm., sampling-time 3,5,8 days (500 ml. flask)
3 - Surface area 43.9 sq.cm., sampling-time 2,4,6,8,10 days (250 ml. flask)

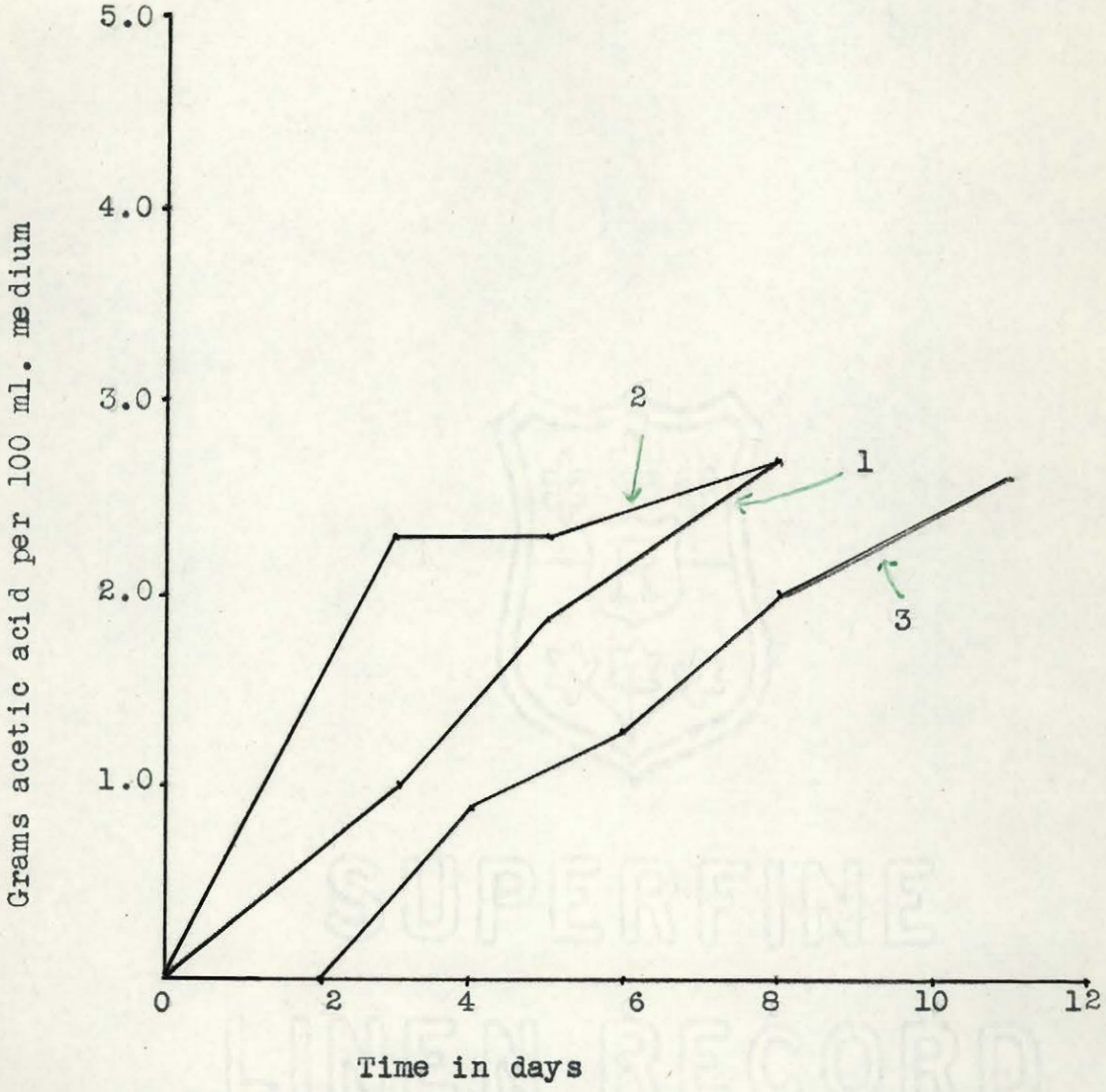


FIGURE 13 - I. THE EFFECT OF VARYING THE SURFACE AREA OF THE INOCULATED YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED. II. A COMPARISON OF EFFICIENCY IN STUDYING THE RATE OF ACID PRODUCTION BY USING DIFFERENT SAMPLING-TIME INTERVALS. ACETOBACTER ACETI 1

Curve 1 - Surface area 43.9 sq.cm., sampling-time 3,5,8 days (250 ml. flask)
2 - Surface area 76.6 sq.cm., sampling-time 3,5,8 days (500 ml. flask)
3 - Surface area 43.9 sq.cm., sampling-time 2,4,6,8,10 days (250 ml. flask)

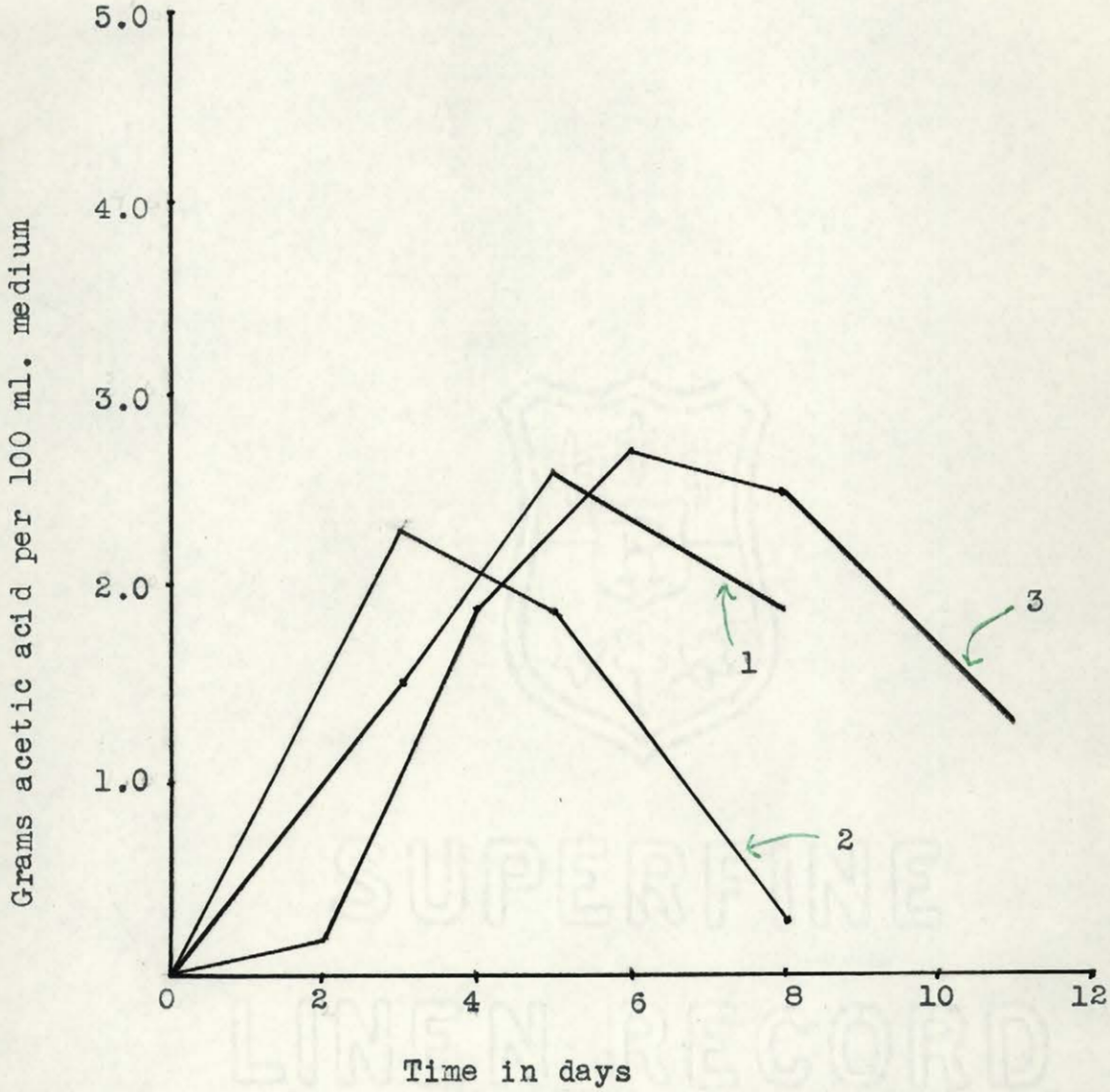


FIGURE 14 - I. THE EFFECT OF VARYING THE SURFACE AREA OF THE INOCULATED YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED. II. A COMPARISON OF EFFICIENCY IN STUDYING THE RATE OF ACID PRODUCTION BY USING DIFFERENT SAMPLING-TIME INTERVALS. ACETOBACTER ACETI 2

Curve 1 - Surface area 43.9 sq.cm., sampling-time 3,5,8 days (250 ml. flask)
2 - Surface area 76.6 sq.cm., sampling-time 3,5,8 days (500 ml. flask)
3 - Surface area 43.9 sq.cm., sampling-time 2,4,6,8,10 days (250 ml. flask)

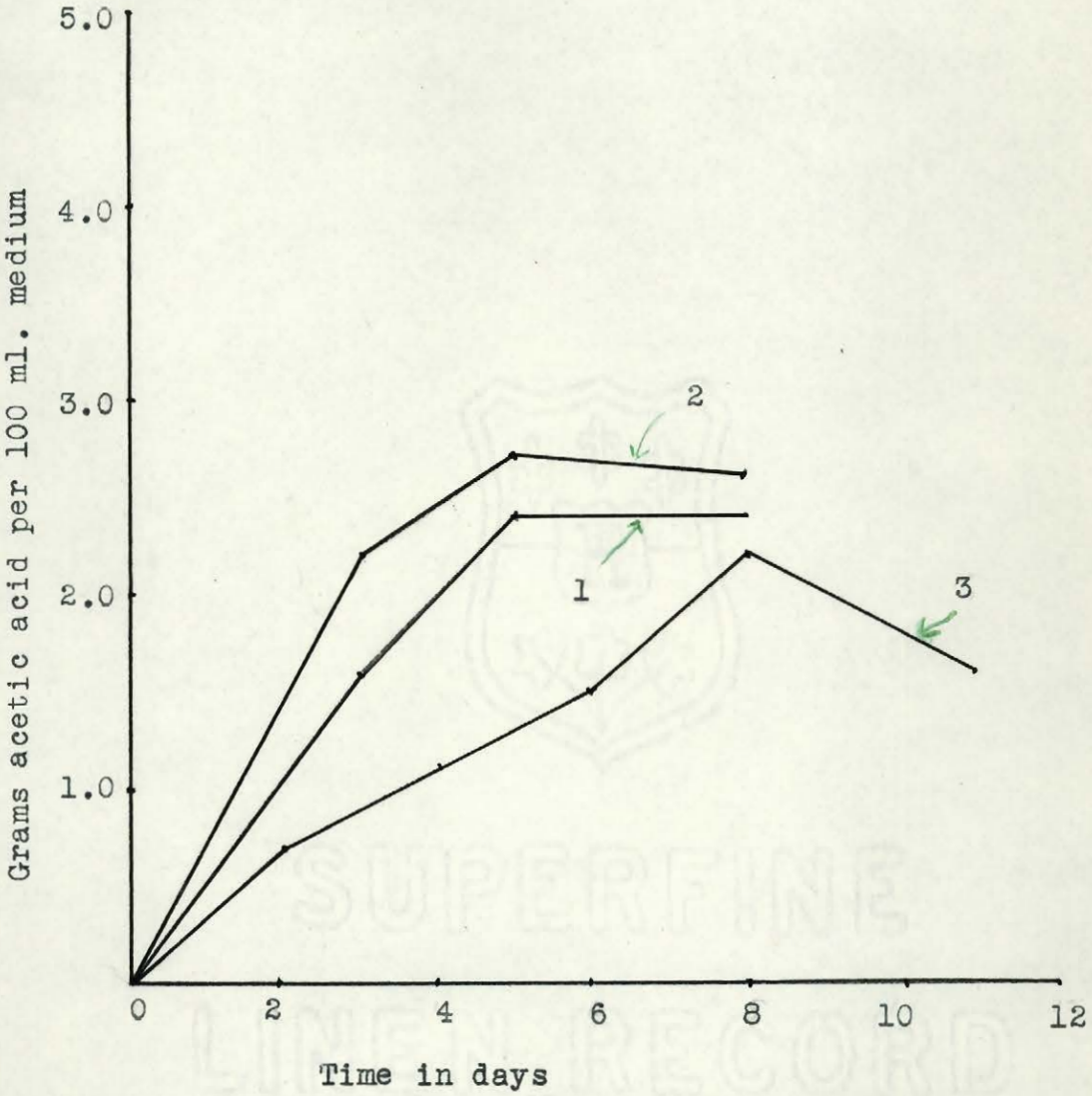


FIGURE 15 - I. THE EFFECT OF VARYING THE SURFACE AREA OF THE INOCULATED YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED. II. A COMPARISON OF EFFICIENCY IN STUDYING THE RATE OF ACID PRODUCTION BY USING DIFFERENT SAMPLING-TIME INTERVALS. ACETOBACTER RANCENS

Curve 1 - Surface area 43.9 sq.cm., sampling-time 3,5,8 days (250 ml. flask)
2 - Surface area 76.6 sq.cm., sampling-time 3,5,8 days (500 ml. flask)
3 - Surface area 43.9 sq.cm., sampling-time 2,4,6,8,10 days (250 ml. flask)

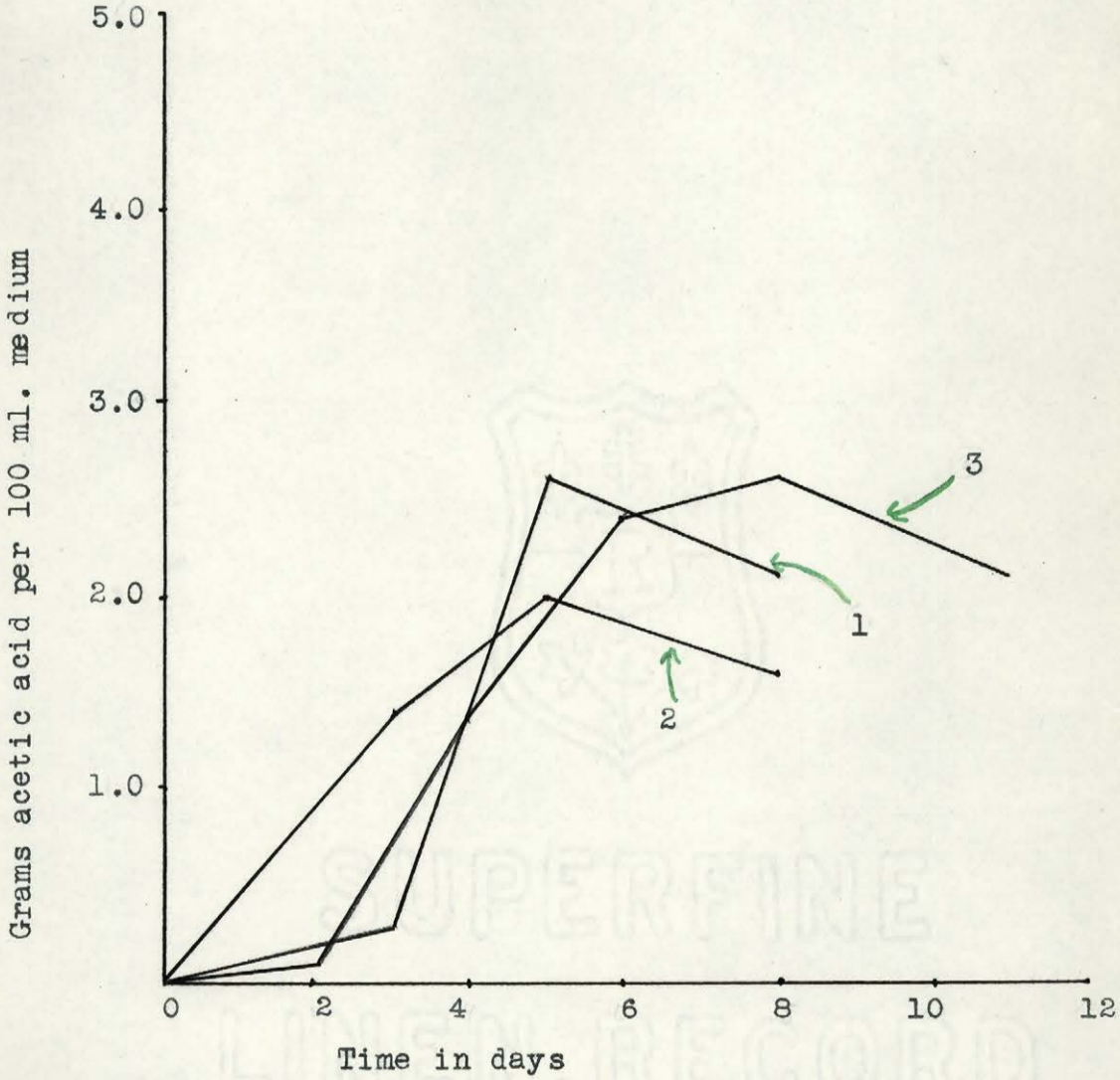


FIGURE 16 - I. THE EFFECT OF VARYING THE SURFACE AREA OF THE INOCULATED YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED. II. A COMPARISON OF EFFICIENCY IN STUDYING THE RATE OF ACID PRODUCTION BY USING DIFFERENT SAMPLING-TIME INTERVALS. ACETOBACTER OXYDANS 1

Curve 1 - Surface area 43.9 sq.cm., sampling-time 3,5,8 days (250 ml. flask)
2 - Surface area 76.6 sq.cm., sampling-time 3,5,8 days (500 ml. flask)
3 - Surface area 43.9 sq.cm., sampling-time 2,4,6,8,10 days (250 ml. flask)

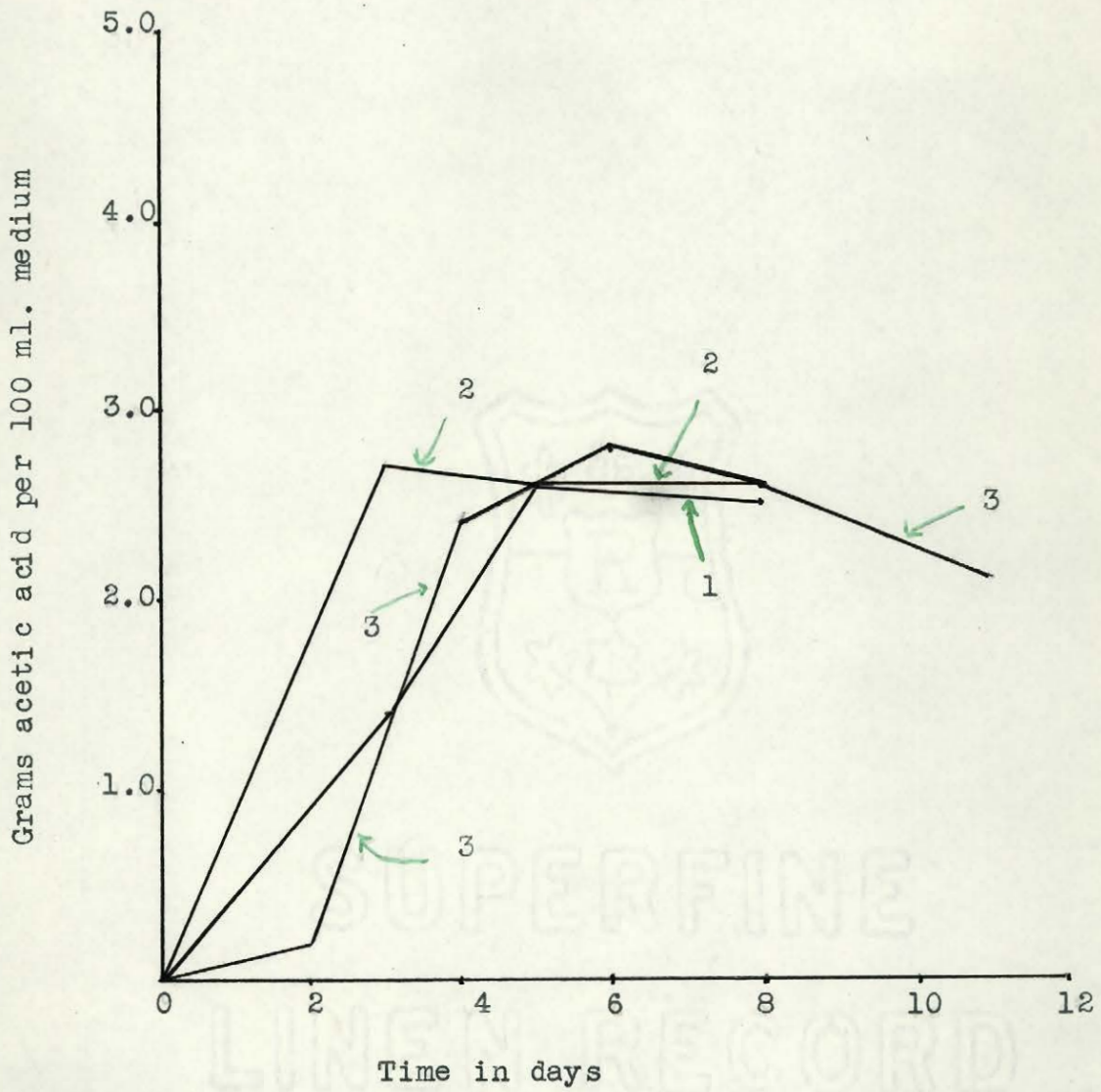


FIGURE 17 - I. THE EFFECT OF VARYING THE SURFACE AREA OF THE INOCULATED YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED. II. A COMPARISON OF EFFICIENCY IN STUDYING THE RATE OF ACID PRODUCTION BY USING DIFFERENT SAMPLING-TIME INTERVALS. ACETOBACTER OXYDANS 2

Curve 1 - Surface area 43.9 sq.cm., sampling-time 3,5,8 days (250 ml. flask)
2 - Surface area 76.6 sq.cm., sampling-time 3,5,8 days (500 ml. flask)
3 - Surface area 43.9 sq.cm., sampling-time 2,4,6,8,10 days (250 ml. flask)

In terms of maximum acid production each organism oxidized ethyl alcohol satisfactorily in 250 ml. Erlenmeyer flasks. Acetobacter ascendens and Acetobacter oxydans 1 produced significantly more acid when fermentations were done in 250 ml. flasks.

Two hundred and fifty milliliter flasks were used during succeeding experiments, because they did not require the extra space needed for 500's and they facilitated the study of the trend in acid production.

iii. The Sampling-Time Intervals of the Fermenting Media

The results of the experiments are recorded in Figures 10 to 17 (pp.151-158), and Appendix Tables VII, X and XIII (pp. 251, 254 and 257).

During fermentation experiments Acetobacter gluconicum produced an insignificant amount of acetic acid; Acetobacter melanogenum 1 produced a maximum yield of 0.27 gm. of acid in 100 ml. of medium in the two, four, six, eight and ten-day sample interval test.

Taking the possible great difference in acid production between the second and third day into consideration, due to the time required for organisms to begin to affect the medium, Acetobacter acetosum, Acetobacter aceti 2 and Acetobacter oxydans 2 each produced somewhat similar curves of acetic acid production with both variations. Acetobacter aceti 1 and Acetobacter rancens produced a decided lag in acid production in the two, four, six, eight and ten-day sample interval test.

Each organism produced insignificantly different maximum amounts of acetic acid with variations in the sampling-time intervals. Acetobacter ascendens, Acetobacter aceti 1, Acetobacter rancens and Acetobacter oxydans 1 each produced a maximum amount of acetic acid in a shorter interval of time during the three, five and eight-day sample interval test.

The two, four, six, eight and ten-day sample interval was considered optimum because the shorter interval of time between sampling allowed for a better study of the rate at which acetic acid was produced. Where necessary the acid production caught up with the eight-day acid production within the ten days. When a maximum amount of acetic acid was produced in eleven days, the results in ten days were still insignificantly different to those of the other tests as shown on the figures.

iv. The Initial Ethyl Alcohol Content of the Media

The results of the experiments are recorded in Figures 18 to 26 (pp.161-169), and Appendix Tables X to XII (pp.254-256) and XIV (p.258).

During fermentation experiments Acetobacter gluconicum produced an insignificant amount of acetic acid; Acetobacter melanogenum 1 produced a maximum yield of 3.59 gm. of acid per 100 ml. in a medium containing 6 per cent alcohol in ten days, and 0.27 gm. in a medium containing 3 per cent in eleven days.

All other organisms produced a varying degree of lag in acetic acid production when the fermentation medium initially contained 6 per cent ethyl alcohol instead of 3 per cent. Using a

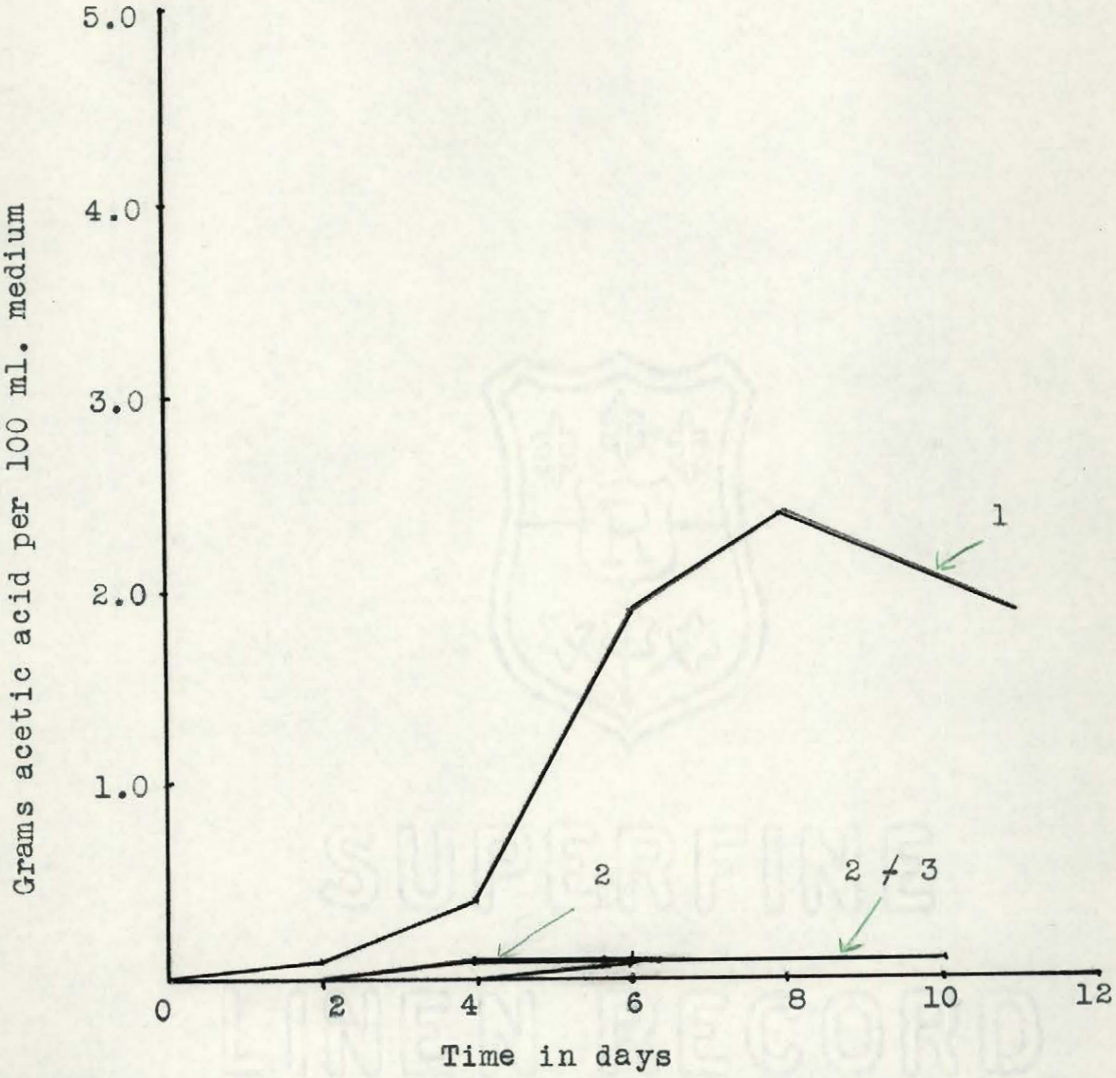


FIGURE 18 - THE EFFECT OF VARYING THE INITIAL ETHYL ALCOHOL CONTENT OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER ACETOSUM

Curve 1 - Ethyl alcohol content 3 per cent
2 - Ethyl alcohol content 6 per cent
3 - Ethyl alcohol content 9 per cent

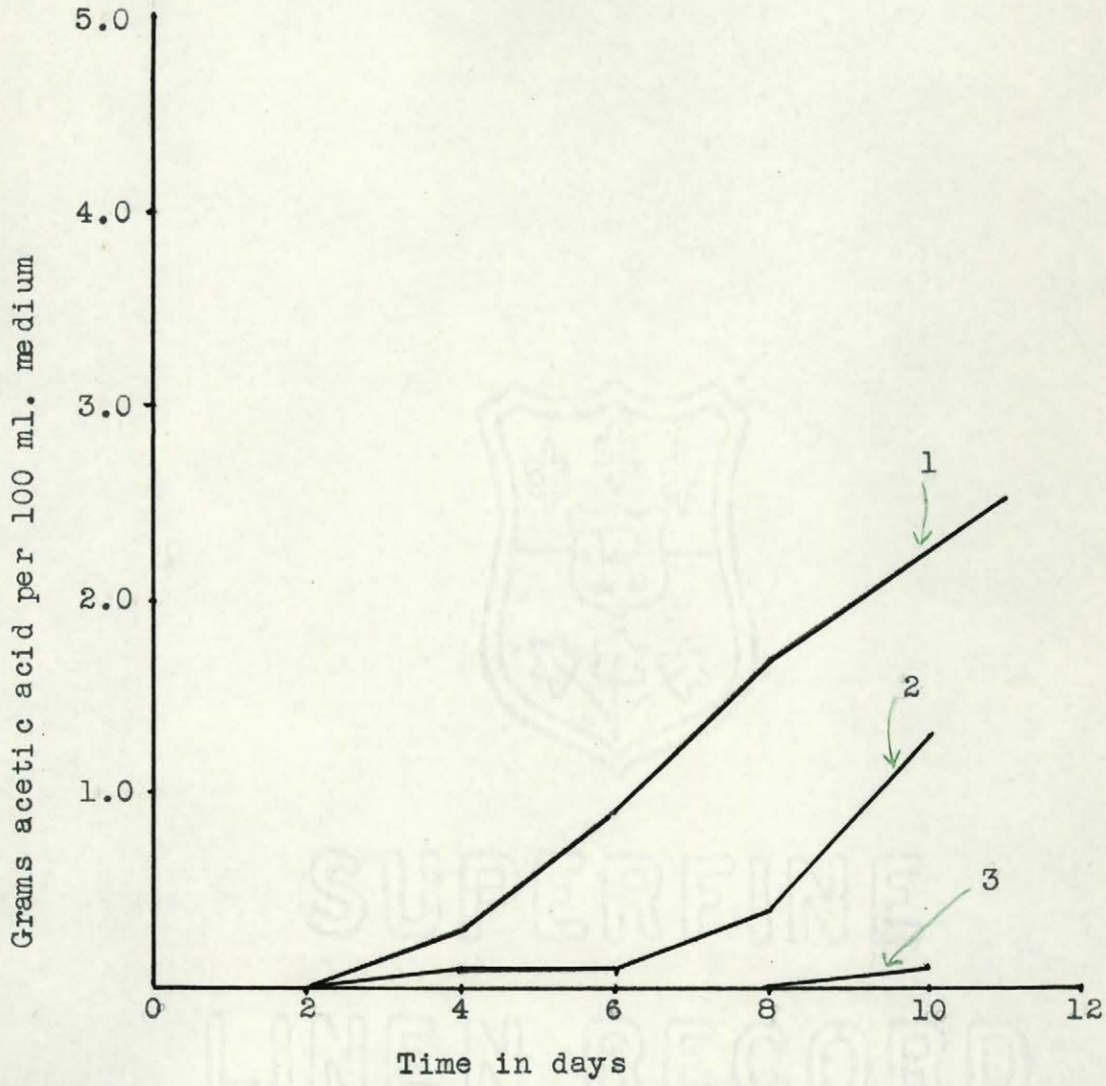


FIGURE 19 - THE EFFECT OF VARYING THE INITIAL ETHYL ALCOHOL CONTENT OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER ASCENDENS

Curve 1 - Ethyl alcohol content 3 per cent
2 - Ethyl alcohol content 6 per cent
3 - Ethyl alcohol content 9 per cent

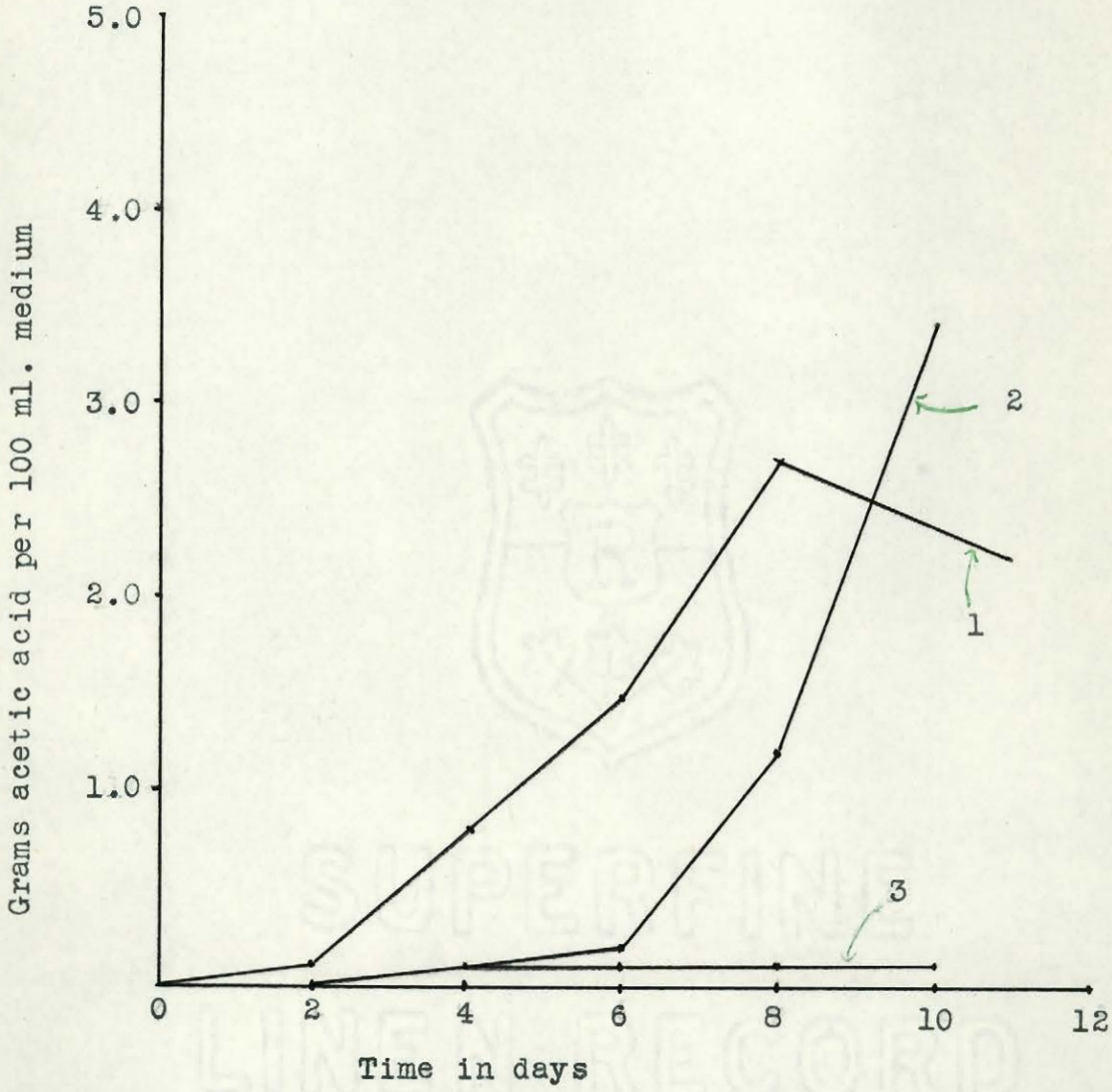


FIGURE 20 - THE EFFECT OF VARYING THE INITIAL ETHYL ALCOHOL CONTENT OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER KÜTZINGIANUM

Curve 1 - Ethyl alcohol content 3 per cent
2 - Ethyl alcohol content 6 per cent
3 - Ethyl alcohol content 9 per cent

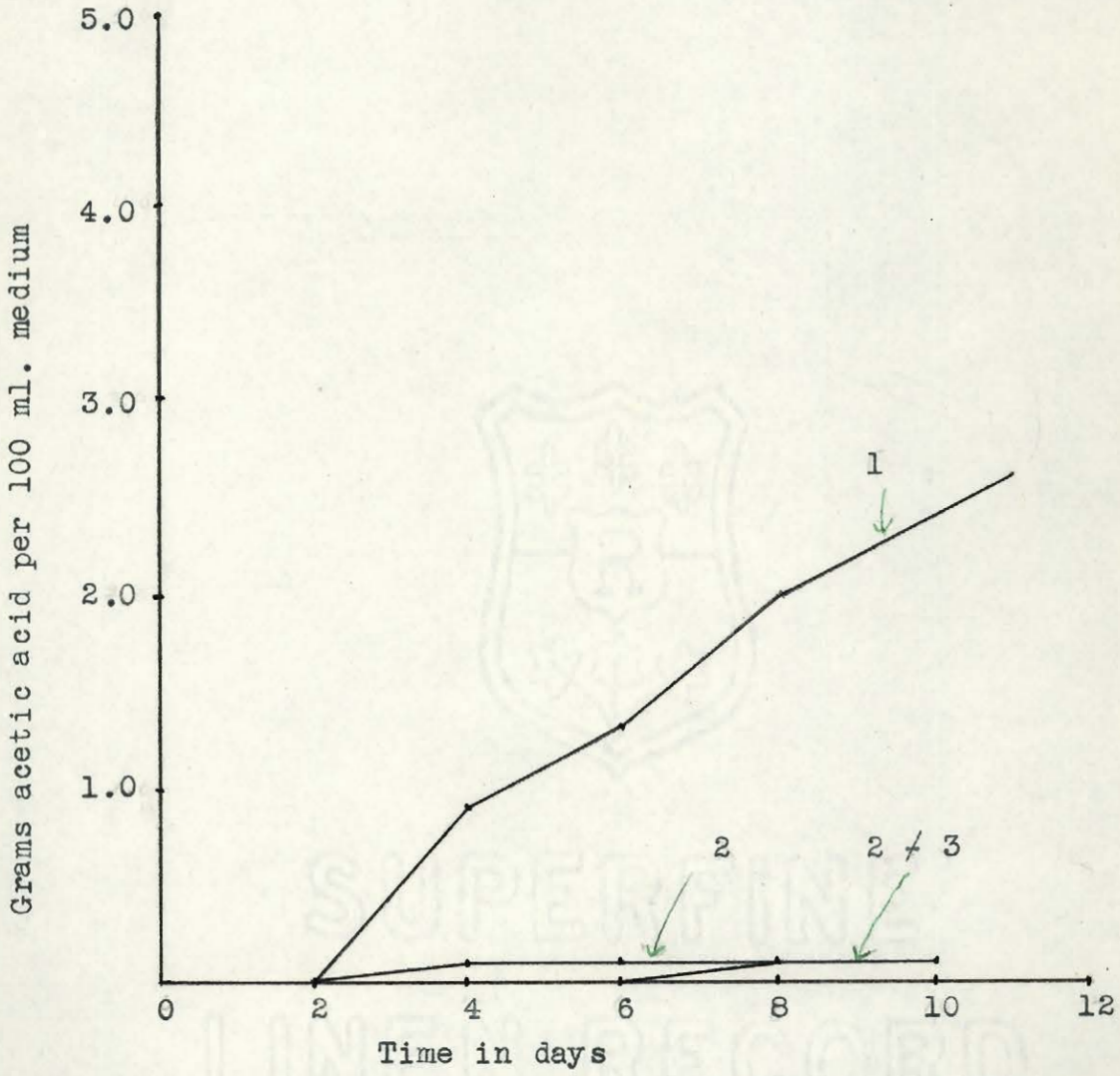


FIGURE 21 - THE EFFECT OF VARYING THE INITIAL ETHYL ALCOHOL CONTENT OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER ACETI 1

Curve 1 - Ethyl alcohol content 3 per cent
2 - Ethyl alcohol content 6 per cent
3 - Ethyl alcohol content 9 per cent

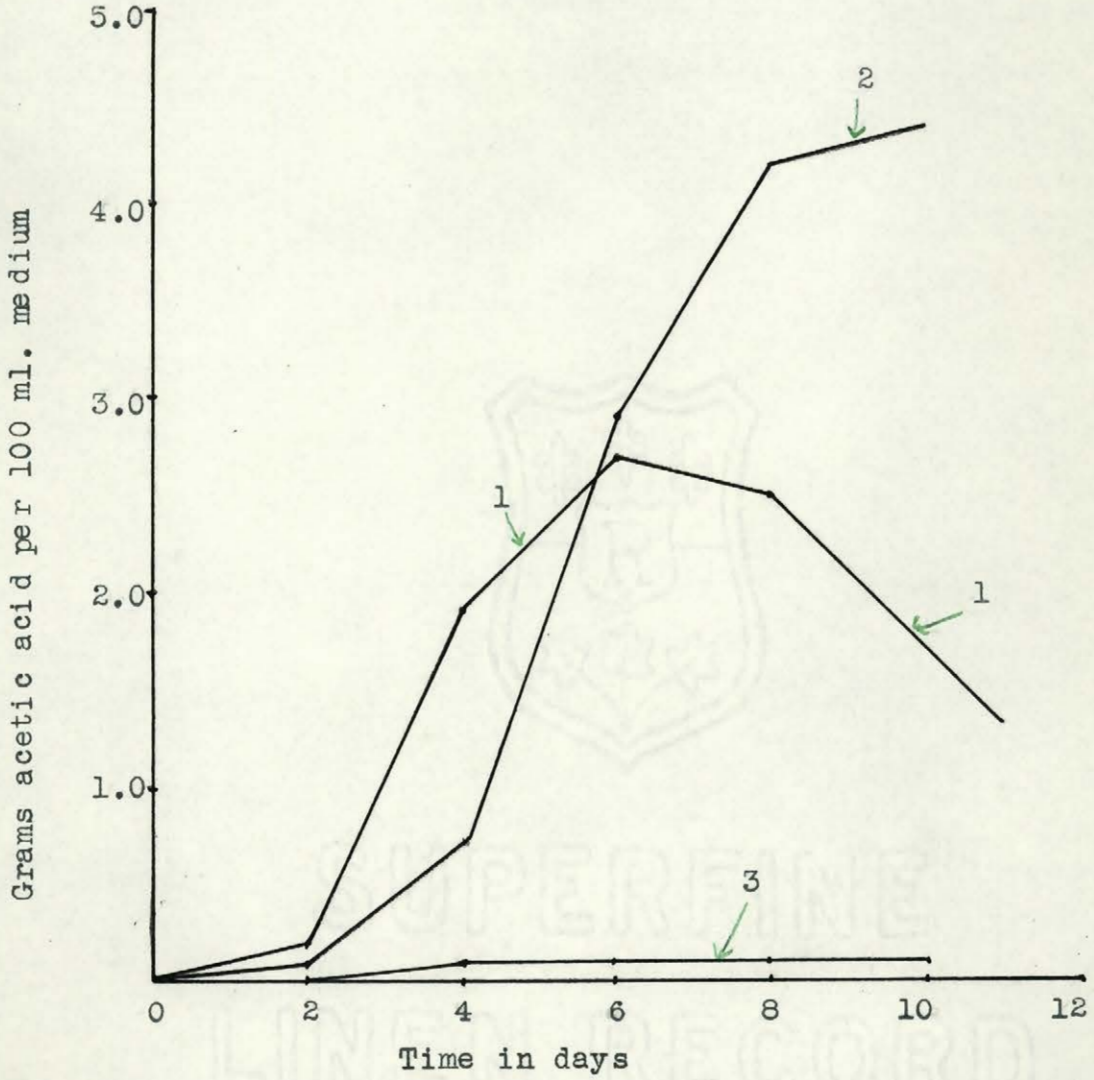


FIGURE 22 - THE EFFECT OF VARYING THE INITIAL ETHYL ALCOHOL CONTENT OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER ACETI 2

Curve 1 - Ethyl alcohol content 3 per cent
2 - Ethyl alcohol content 6 per cent
3 - Ethyl alcohol content 9 per cent

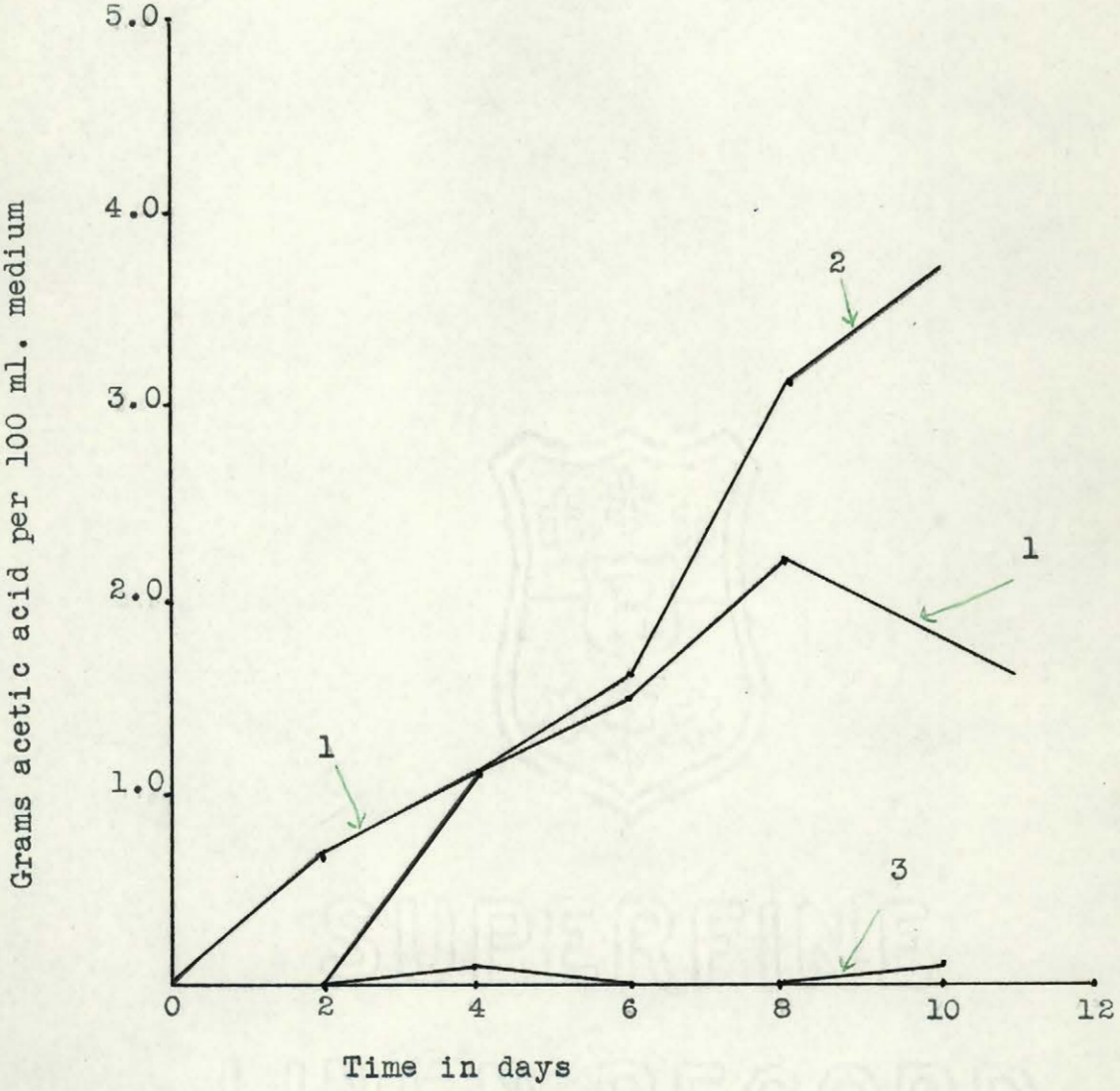


FIGURE 23 - THE EFFECT OF VARYING THE INITIAL ETHYL ALCOHOL CONTENT OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER RANCENS

Curve 1 - Ethyl alcohol content 3 per cent
2 - Ethyl alcohol content 6 per cent
3 - Ethyl alcohol content 9 per cent

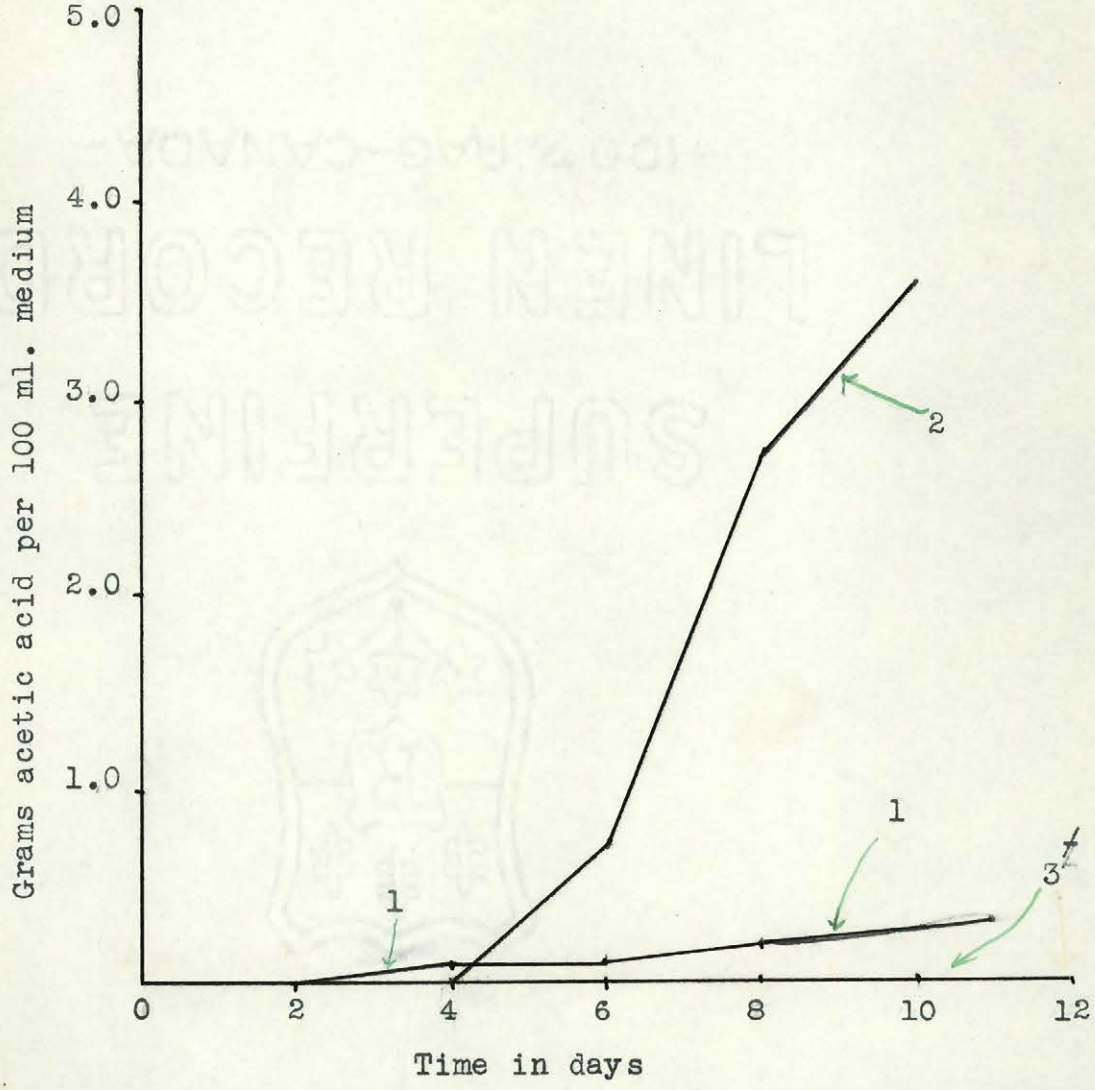


FIGURE 24 - THE EFFECT OF VARYING THE INITIAL ETHYL ALCOHOL CONTENT OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER MELANOGENUM 1

Curve 1 - Ethyl alcohol content 3 per cent
2 - Ethyl alcohol content 6 per cent
3 - Ethyl alcohol content 9 per cent

/ - All readings 0.

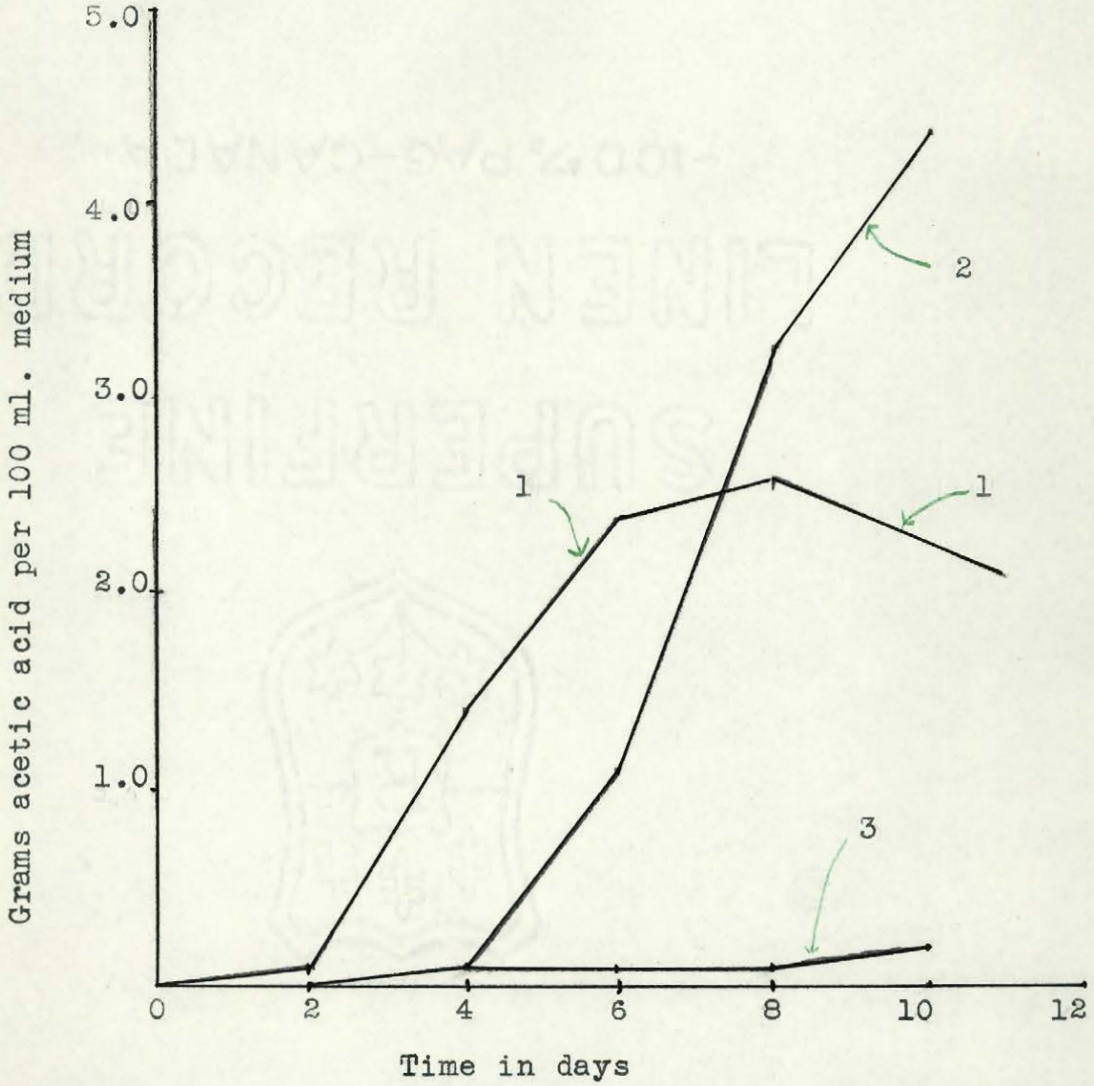


FIGURE 25 - THE EFFECT OF VARYING THE INITIAL ETHYL ALCOHOL CONTENT OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER OXYDANS 1

Curve 1 - Ethyl alcohol content 3 per cent
2 - Ethyl alcohol content 6 per cent
3 - Ethyl alcohol content 9 per cent

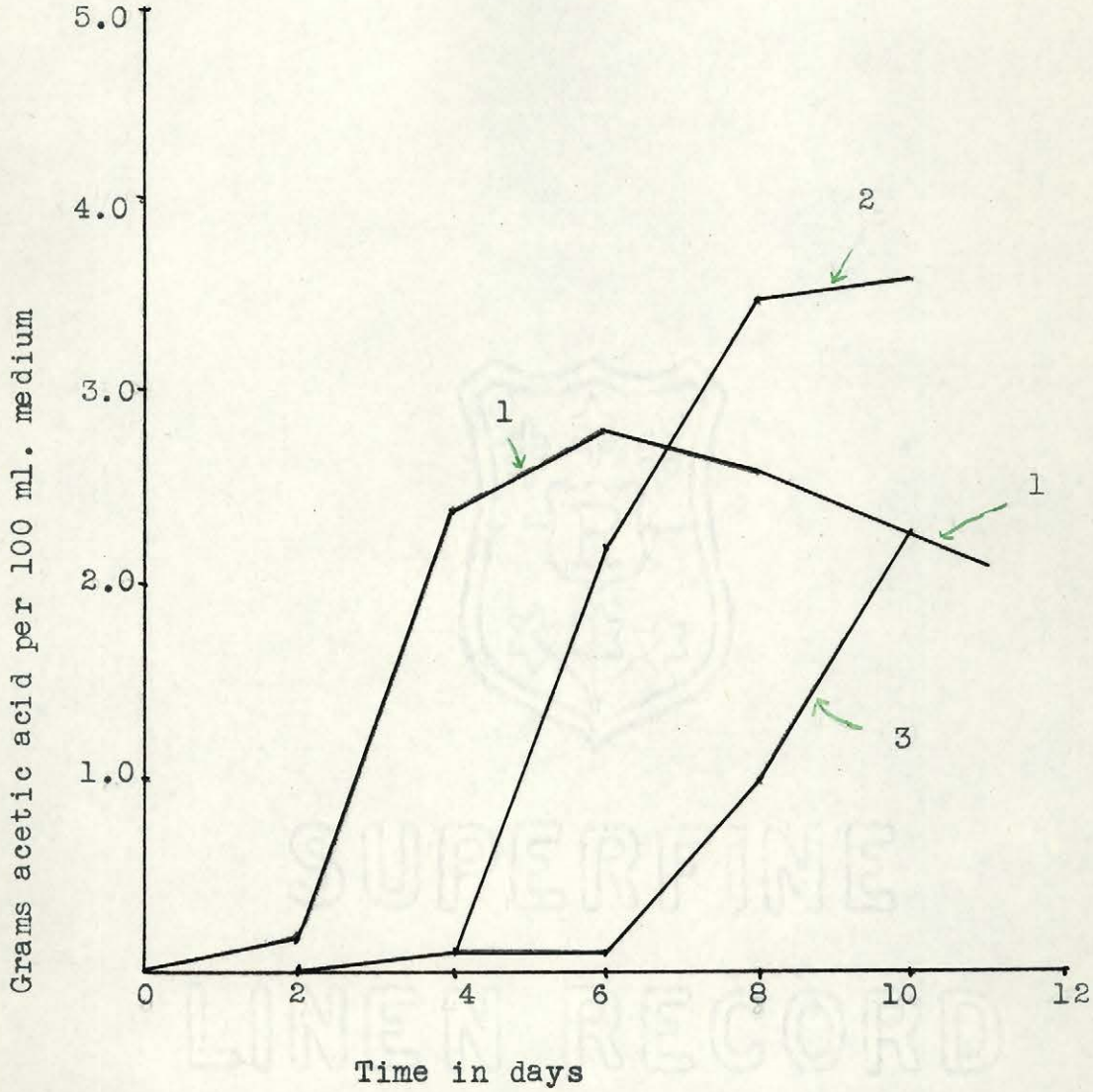


FIGURE 26 - THE EFFECT OF VARYING THE INITIAL ETHYL ALCOHOL CONTENT OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER OXYDANS 2

Curve 1 - Ethyl alcohol content 3 per cent
2 - Ethyl alcohol content 6 per cent
3 - Ethyl alcohol content 9 per cent

fermentation medium containing 9 per cent ethyl alcohol, all organisms produced less than 0.2 gm. acetic acid except Acetobacter oxydans 1 and Acetobacter oxydans 2, which produced a maximum yield of 0.21 and 2.31 gm. of acid per 100 ml. of medium respectively in ten days. Acetobacter oxydans 2 gave a much greater lag in acid production when the initial ethyl alcohol concentration was 9 per cent instead of 6 per cent. Acetobacter acetosum and Acetobacter aceti 1 produced significant quantities of acetic acid only when the medium initially contained 3 per cent alcohol. Acid production with Acetobacter kützingianum, Acetobacter aceti 2, Acetobacter rancens, Acetobacter oxydans 1 and Acetobacter oxydans 2 dropped after eight days when the medium contained 3 per cent alcohol, and went higher when the medium contained 6 per cent.

Acetobacter acetosum, Acetobacter ascendens, and Acetobacter aceti 1 each produced a maximum amount of acetic acid when the medium initially contained 3 per cent ethyl alcohol. Acetobacter kützingianum, Acetobacter aceti 2, Acetobacter rancens, Acetobacter oxydans 1 and Acetobacter oxydans 2 each produced a maximum amount when the medium contained 6 per cent ethyl alcohol.

By calculation from molecular weights of ethyl alcohol and acetic acid, and the specific gravity of 95 per cent ethyl alcohol, acetic acid is produced with 100 per cent efficiency if the maximum yield is 3.21 gm. and 6.42 gm. per

100 ml. in medium containing 3 and 6 per cent alcohol respectively. The maximum amount of acid produced during the study of the effect of 3 per cent alcohol was 2.78 gm., and Acetobacter oxydans 2 produced that quantity; the amount represented 86.6 per cent of the theoretically possible yield. The maximum produced during the study of the effect of 6 per cent alcohol was 4.42 gm., and Acetobacter aceti 2 produced that quantity; the amount represented 68.8 per cent of the theoretical yield.

v. The Initial pH of the Media

The results of the experiments are recorded in Figures 27A to 36B (ppl72-189), and Appendix Tables XV to XXII (pp.259-266).

Acetobacter gluconicum produced a maximum yield of 1.58 gm. of acetic acid per 100 ml. of medium when the initial pH was 5.5 in twelve days, and insignificant amounts when media were adjusted to other pH's. As stated in the results of the effect of alcohol content of the medium, Acetobacter melanogenum 1 produced a good acetic acid yield only when the pH of the medium was adjusted to 6.0.

Each of the other organisms tended to produce an individual type of curve for the rate of acetic acid production at all the pH's of the medium studied; there did seem to be more uniformity in the curves at pH 4.0 to 5.5 than at 5.5 to 7.0. Acetobacter acetosum, Acetobacter ascendens, Acetobacter kützingianum, Acetobacter oxydans 1 and Acetobacter oxydans 2 tended to produce more acid at the four-day interval when the initial pH of the medium was between 4.0 and 5.5 than when the initial pH of

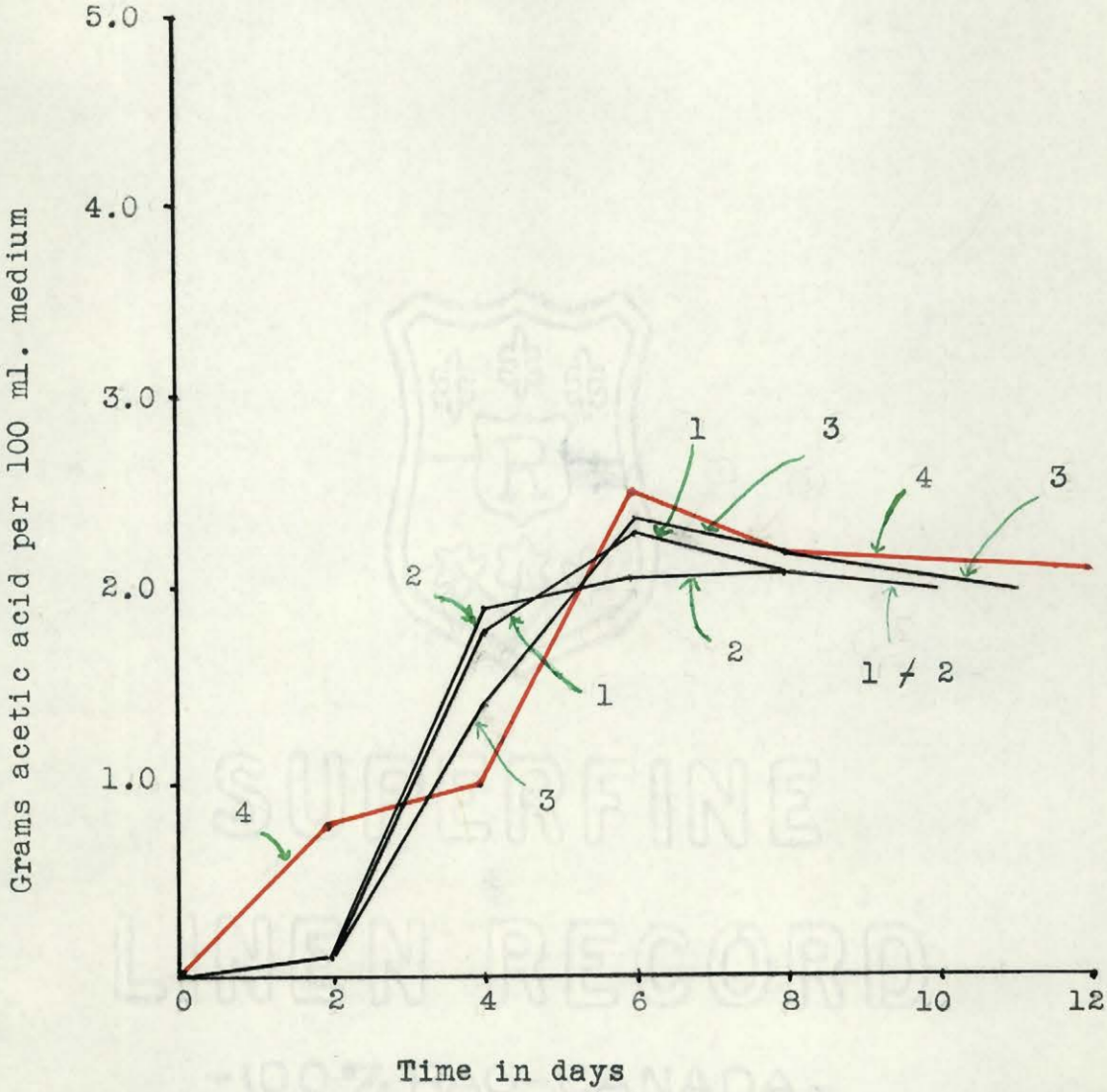


FIGURE 27A - THE EFFECT OF VARYING THE INITIAL pH OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER ACETOSUM

Curve 1 - pH 4.0
Curve 2 - pH 4.5
3 - pH 5.0
4 - pH 5.5

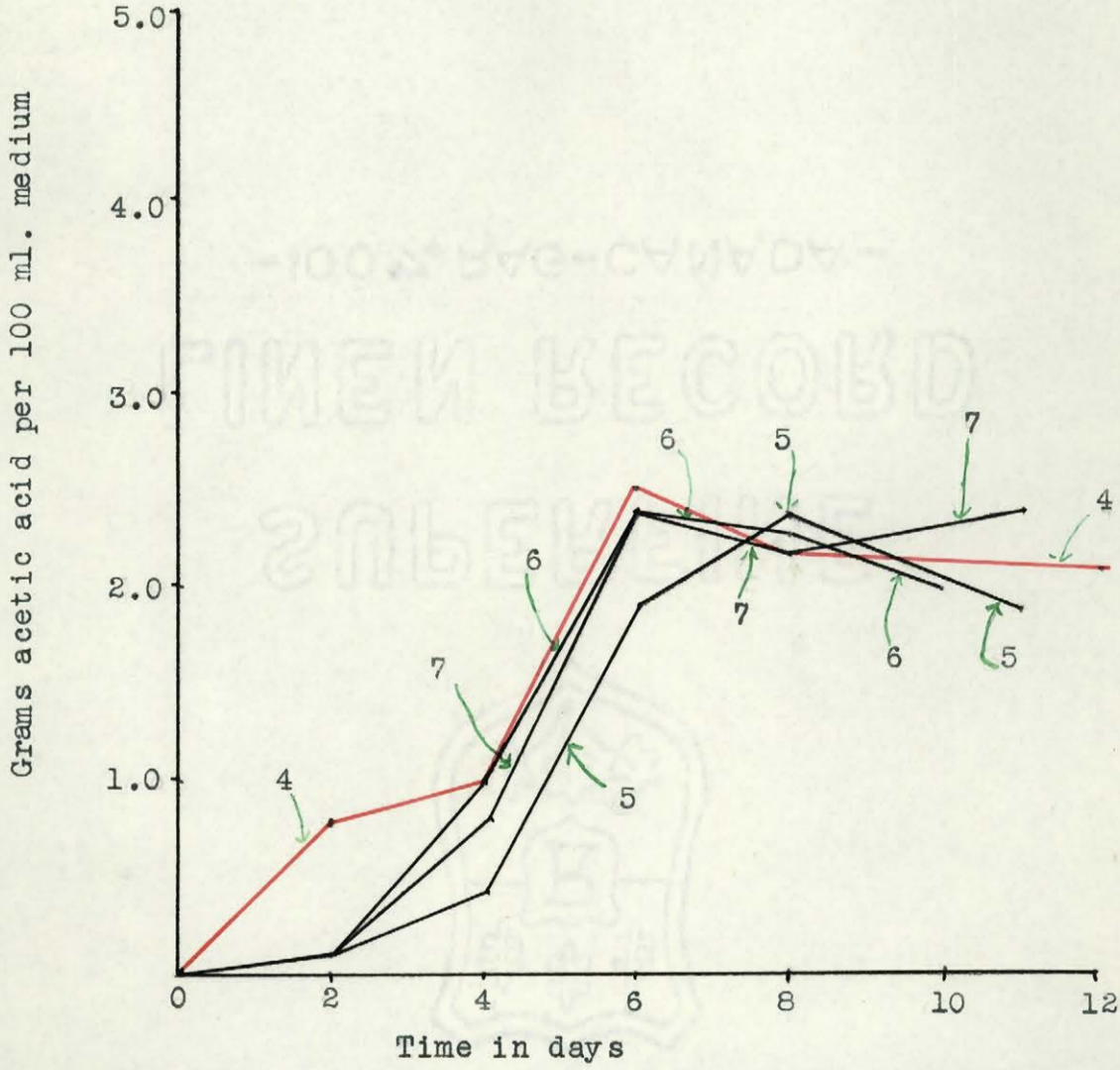


FIGURE 27B - THE EFFECT OF VARYING THE INITIAL pH OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER ACETOSUM

Curve 4 - pH 5.5
5 - pH 6.0
6 - pH 6.5
7 - pH 7.0

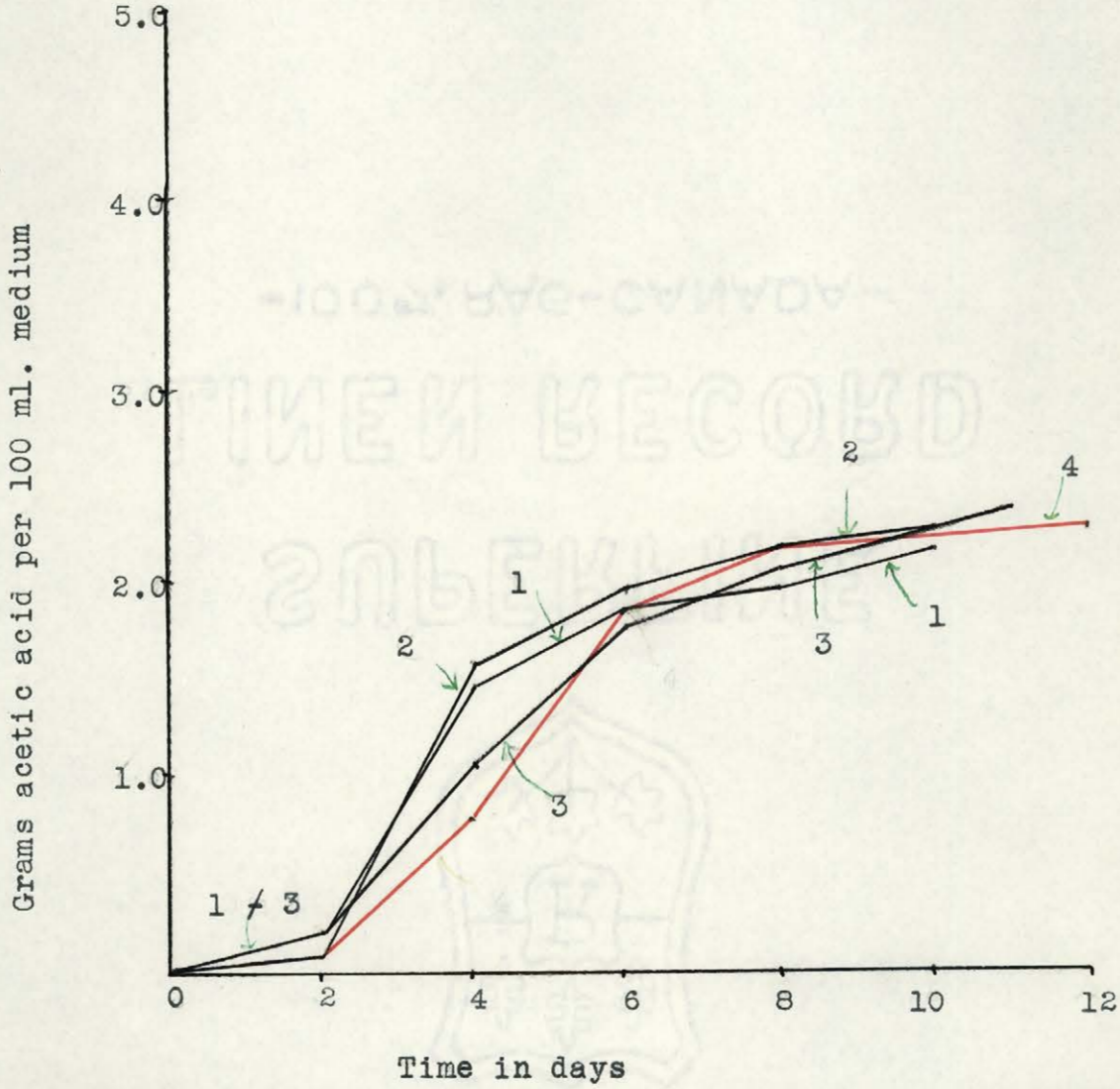


FIGURE 28A - THE EFFECT OF VARYING THE INITIAL pH OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER ASCENDENS

Curve 1 - pH 4.0
2 - pH 4.5
3 - pH 5.0
4 - pH 5.5

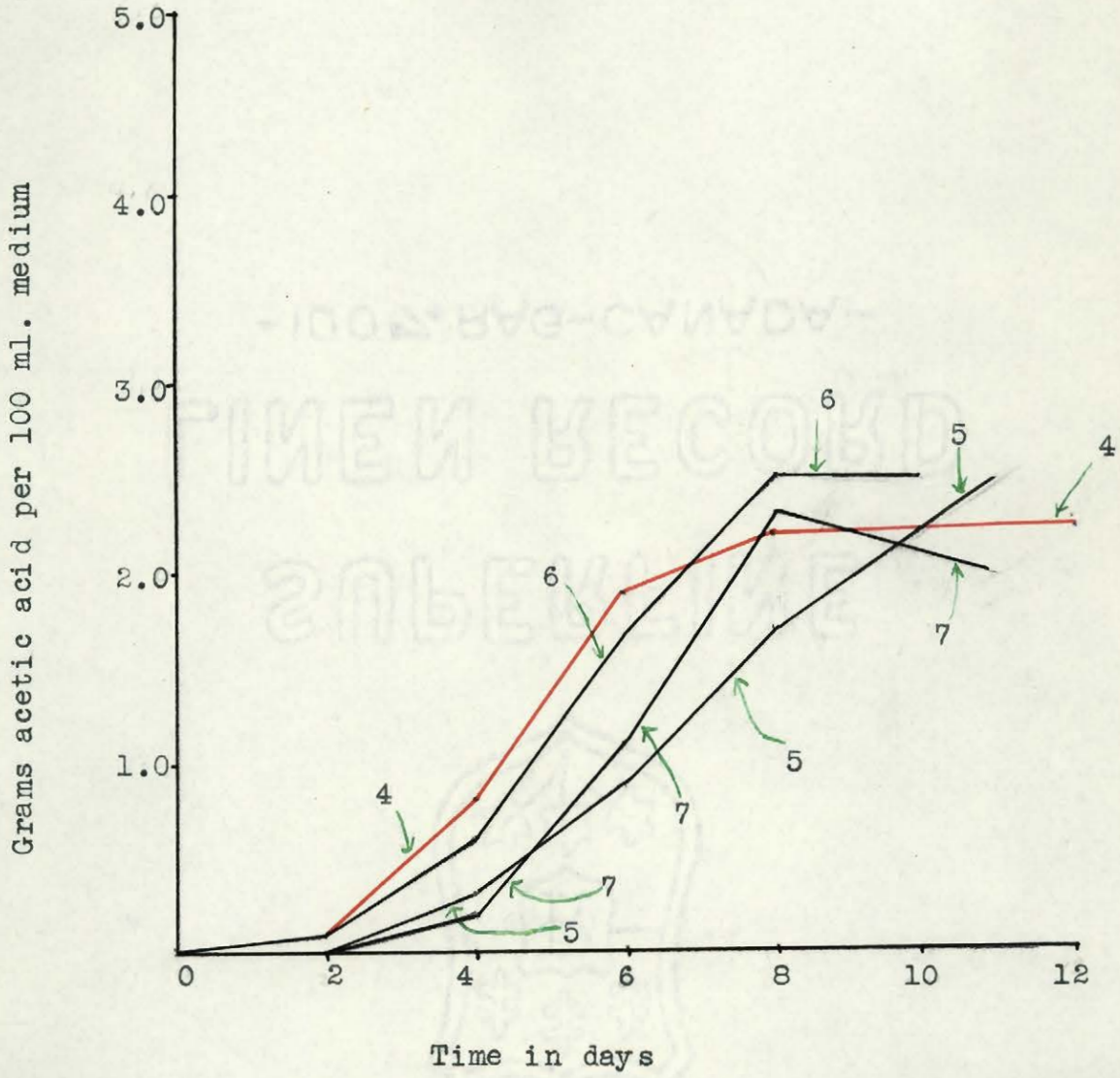


FIGURE 28B - THE EFFECT OF VARYING THE INITIAL pH OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER ASCENDENS

Curve 4 - pH 5.5
5 - pH 6.0
6 - pH 6.5
7 - pH 7.0

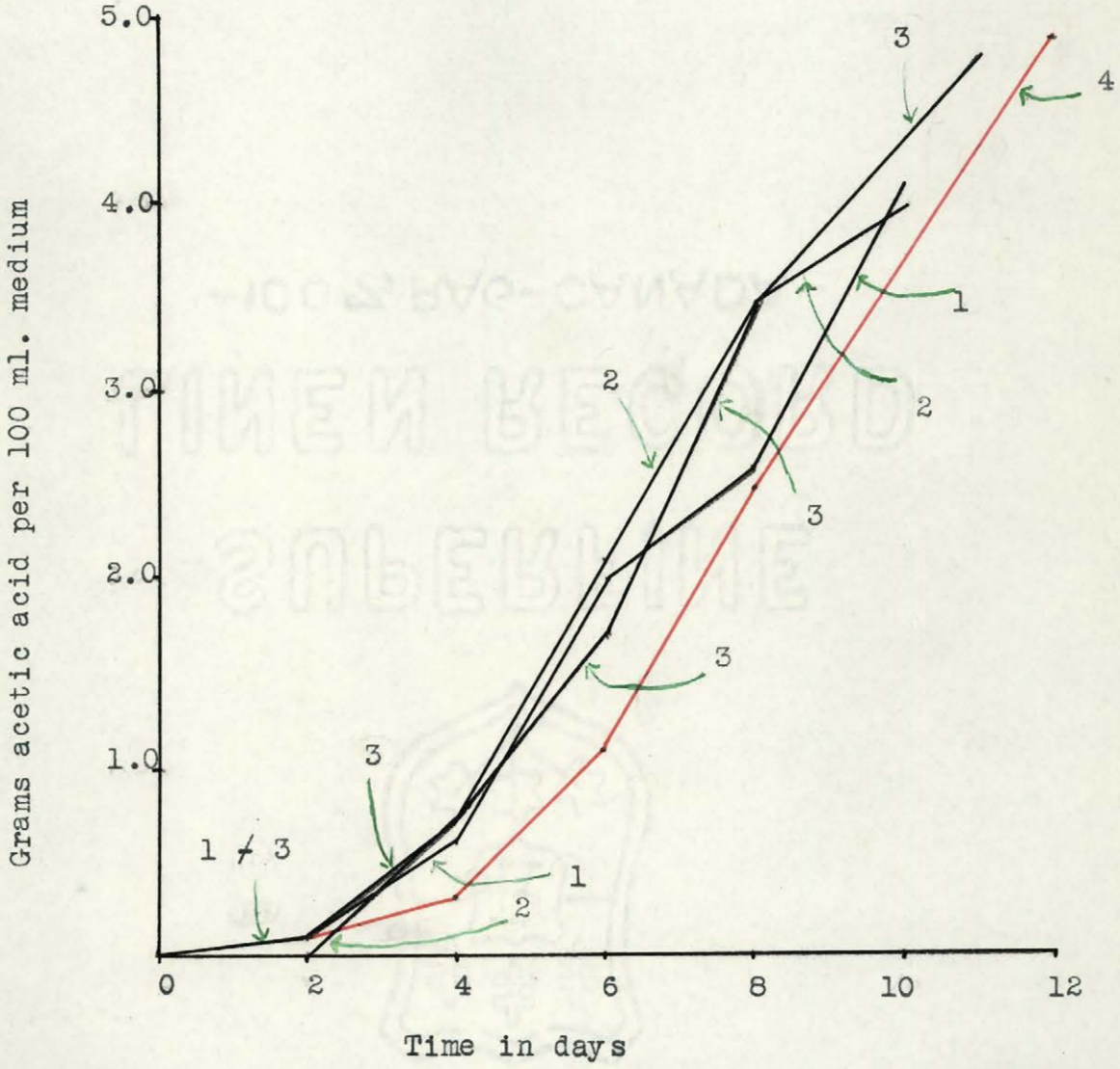


FIGURE 29A - THE EFFECT OF VARYING THE INITIAL pH OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER KUTZINGIANUM

Curve 1 - pH 4.0
2 - pH 4.5
3 - pH 5.0
4 - pH 5.5

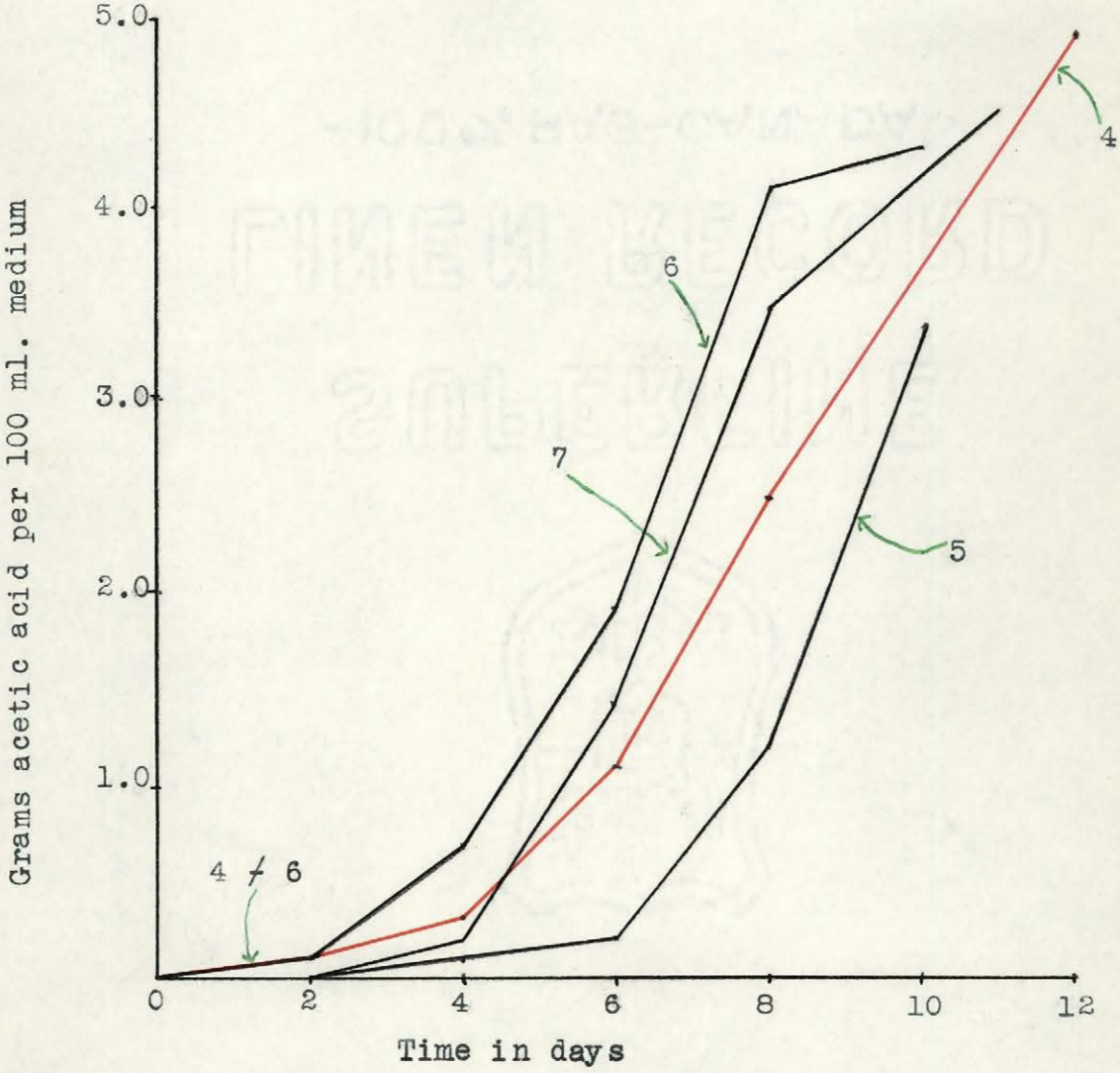


FIGURE 29B - THE EFFECT OF VARYING THE INITIAL pH OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER KÜTZINGIANUM

Curve 4 - pH 5.5
5 - pH 6.0
6 - pH 6.5
7 - pH 7.0

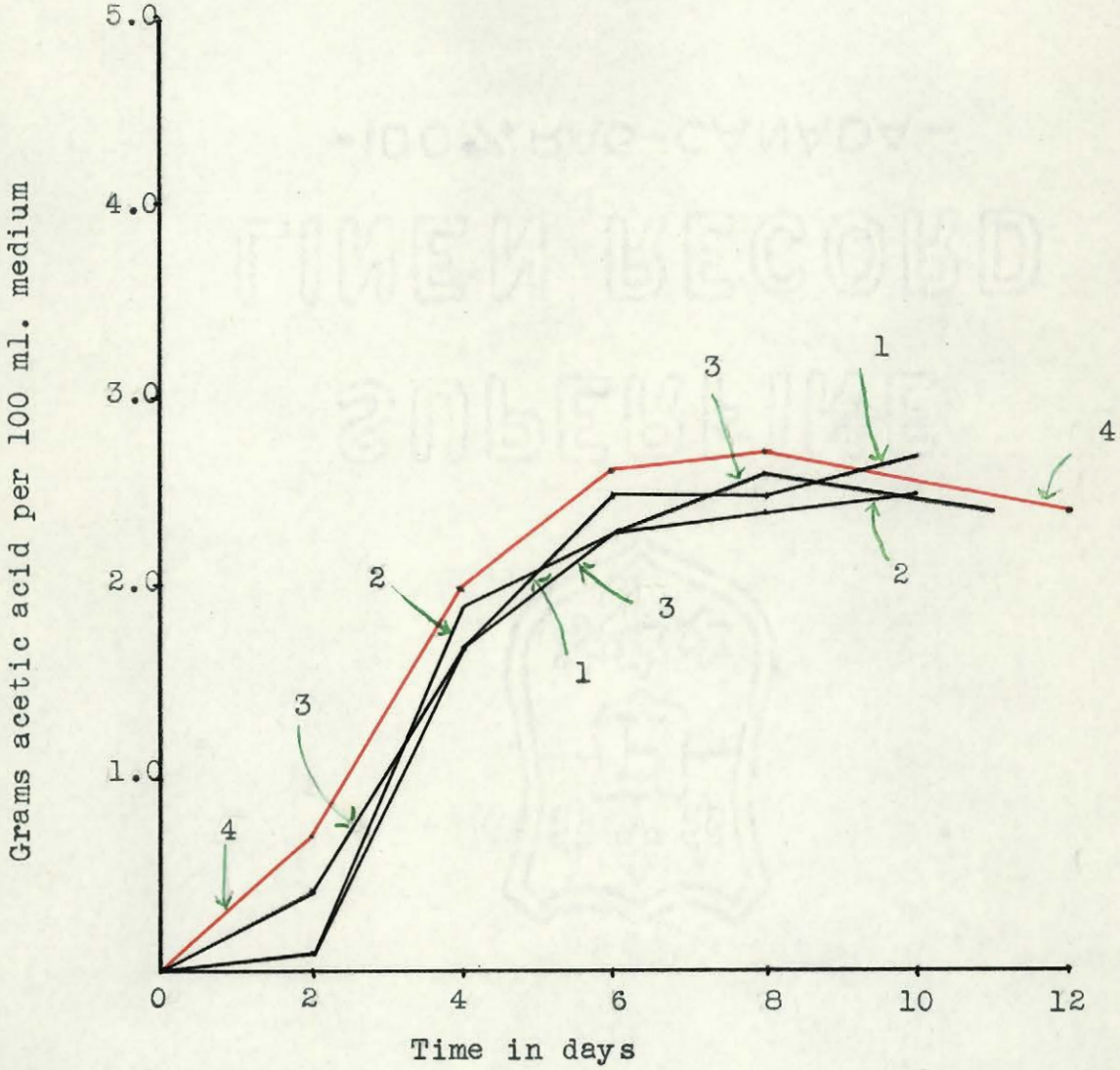


FIGURE 30A - THE EFFECT OF VARYING THE INITIAL pH OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER ACETI 1

Curve 1 - pH 4.0
2 - pH 4.5
3 - pH 5.0
4 - pH 5.5

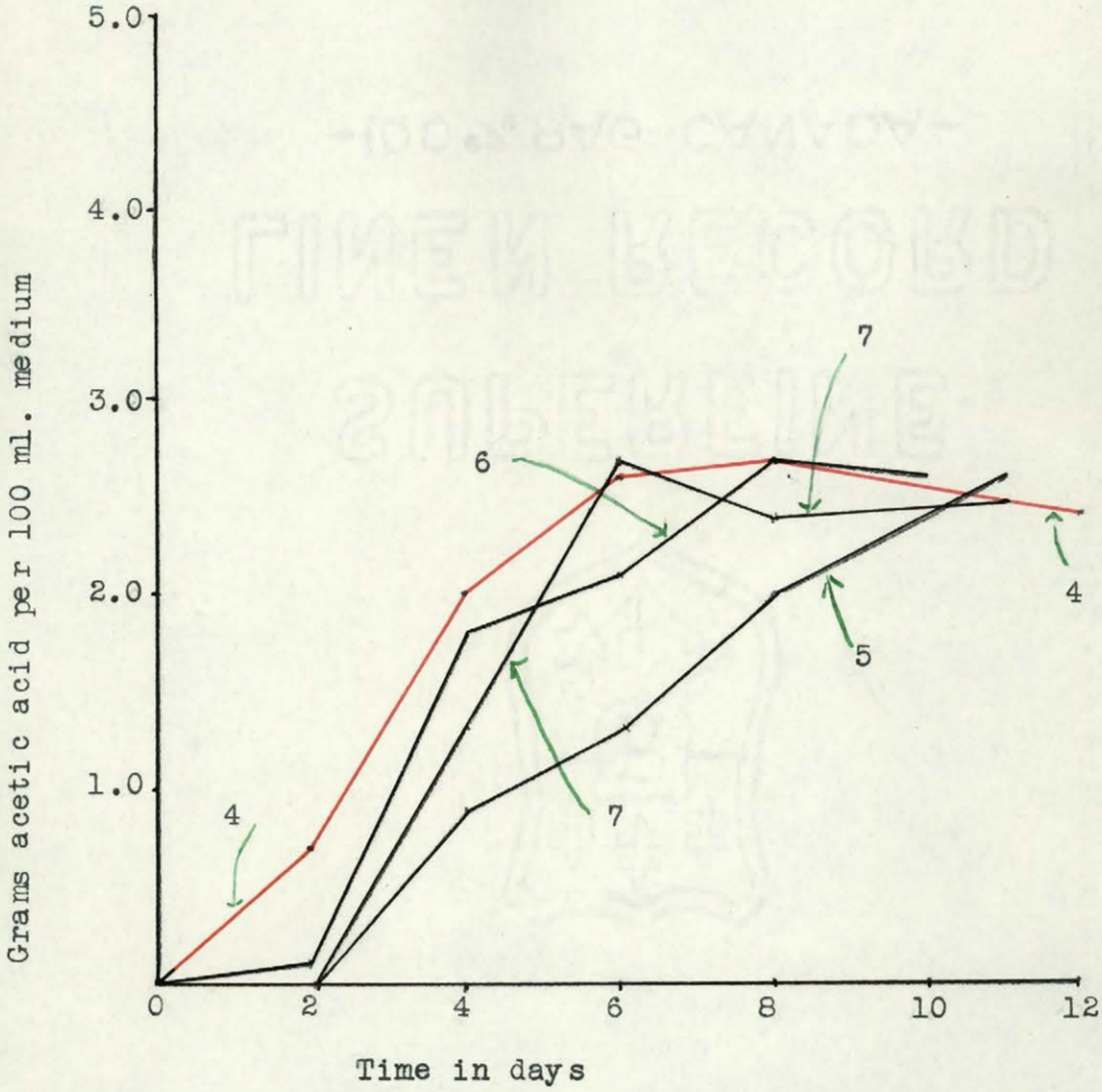


FIGURE 30B - THE EFFECT OF VARYING THE INITIAL pH OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER ACETI 1

Curve 4 - pH 5.5
5 - pH 6.0
6 - pH 6.5
7 - pH 7.0

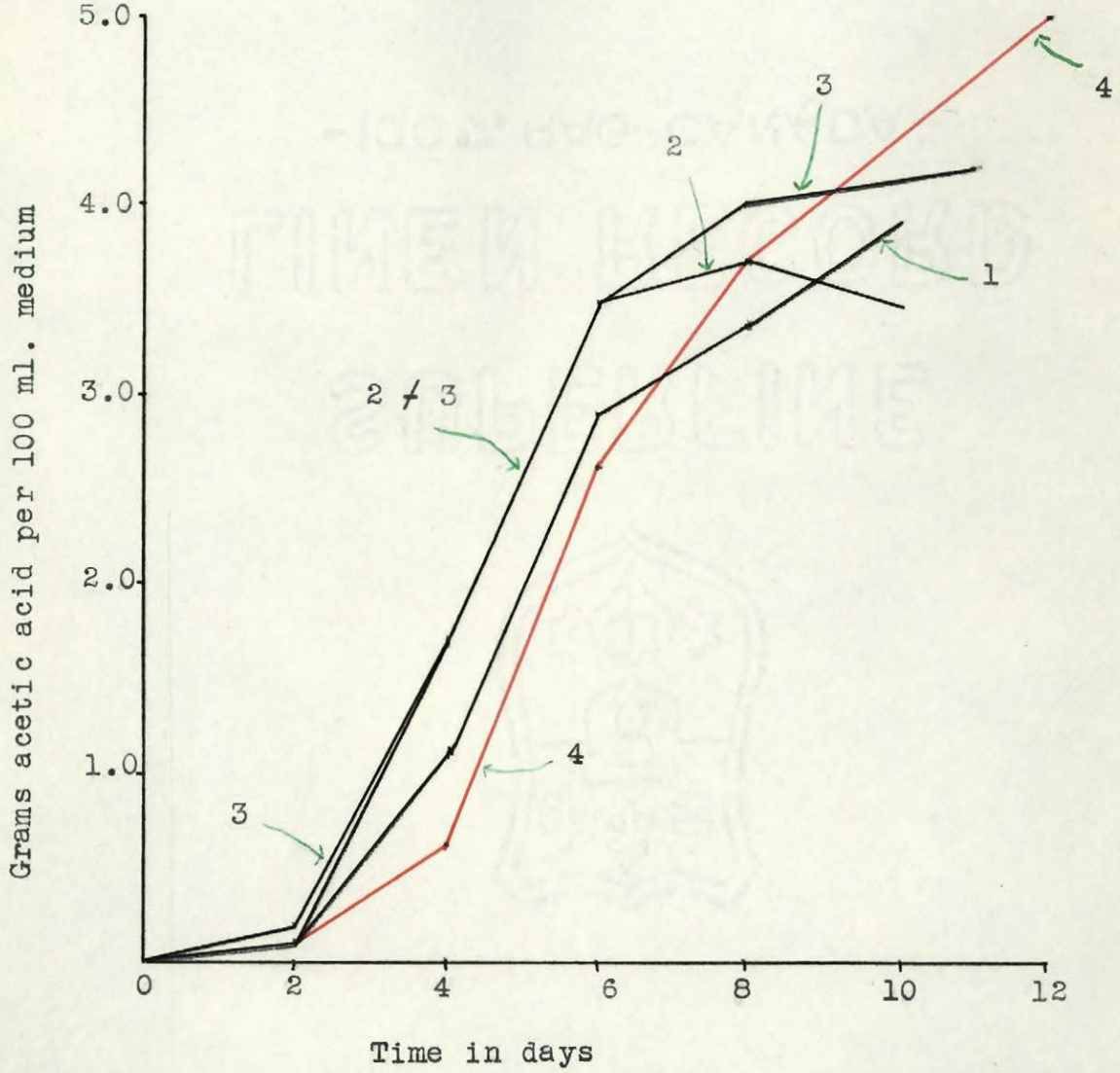


FIGURE 31A - THE EFFECT OF VARYING THE INITIAL pH OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER ACETI 2

Curve 1 - pH 4.0
2 - pH 4.5
3 - pH 5.0
4 - pH 5.5

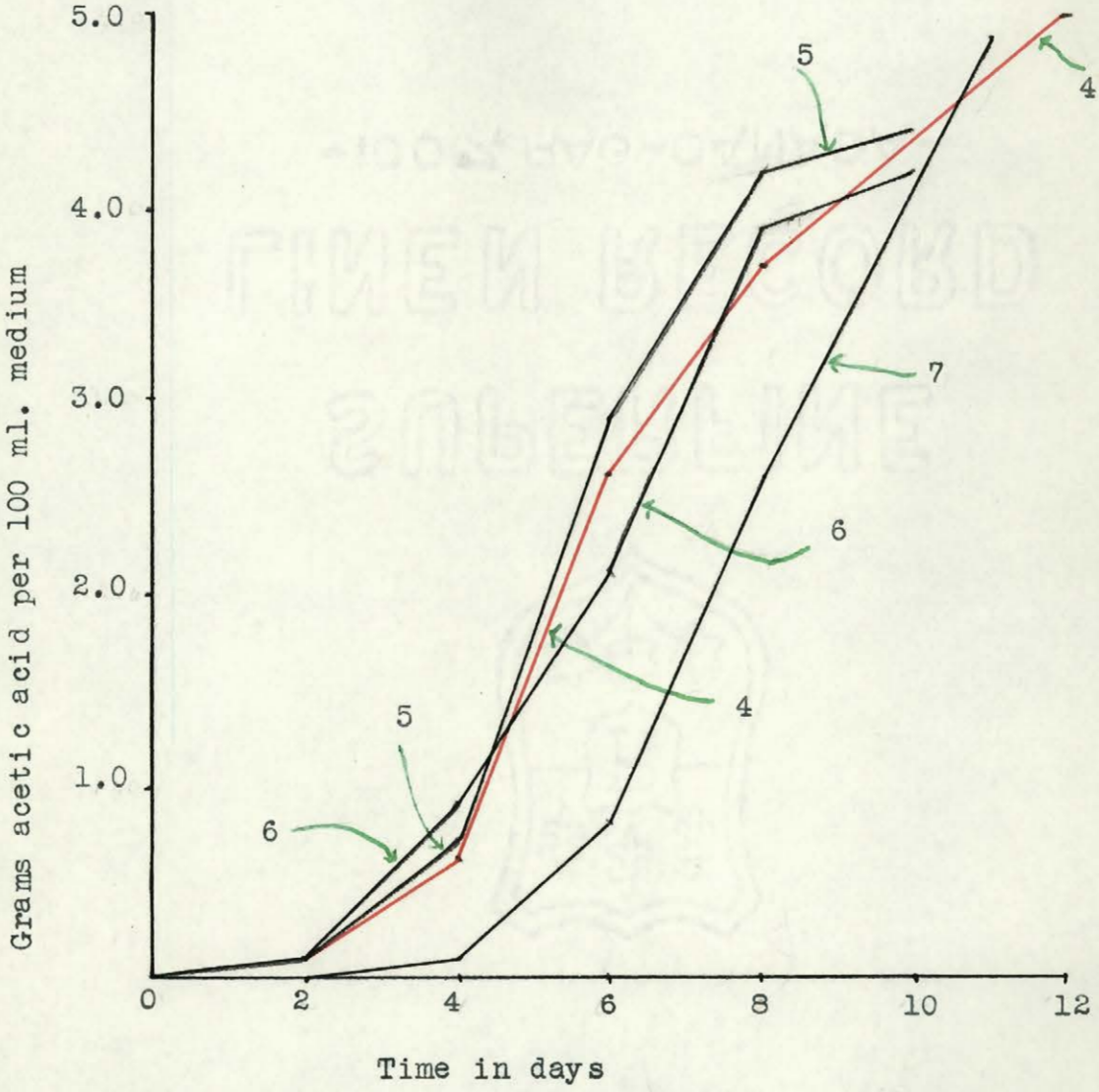


FIGURE 31B - THE EFFECT OF VARYING THE INITIAL pH OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER ACETI 2

Curve 4 - pH 5.5
5 - pH 6.0
6 - pH 6.5
7 - pH 7.0

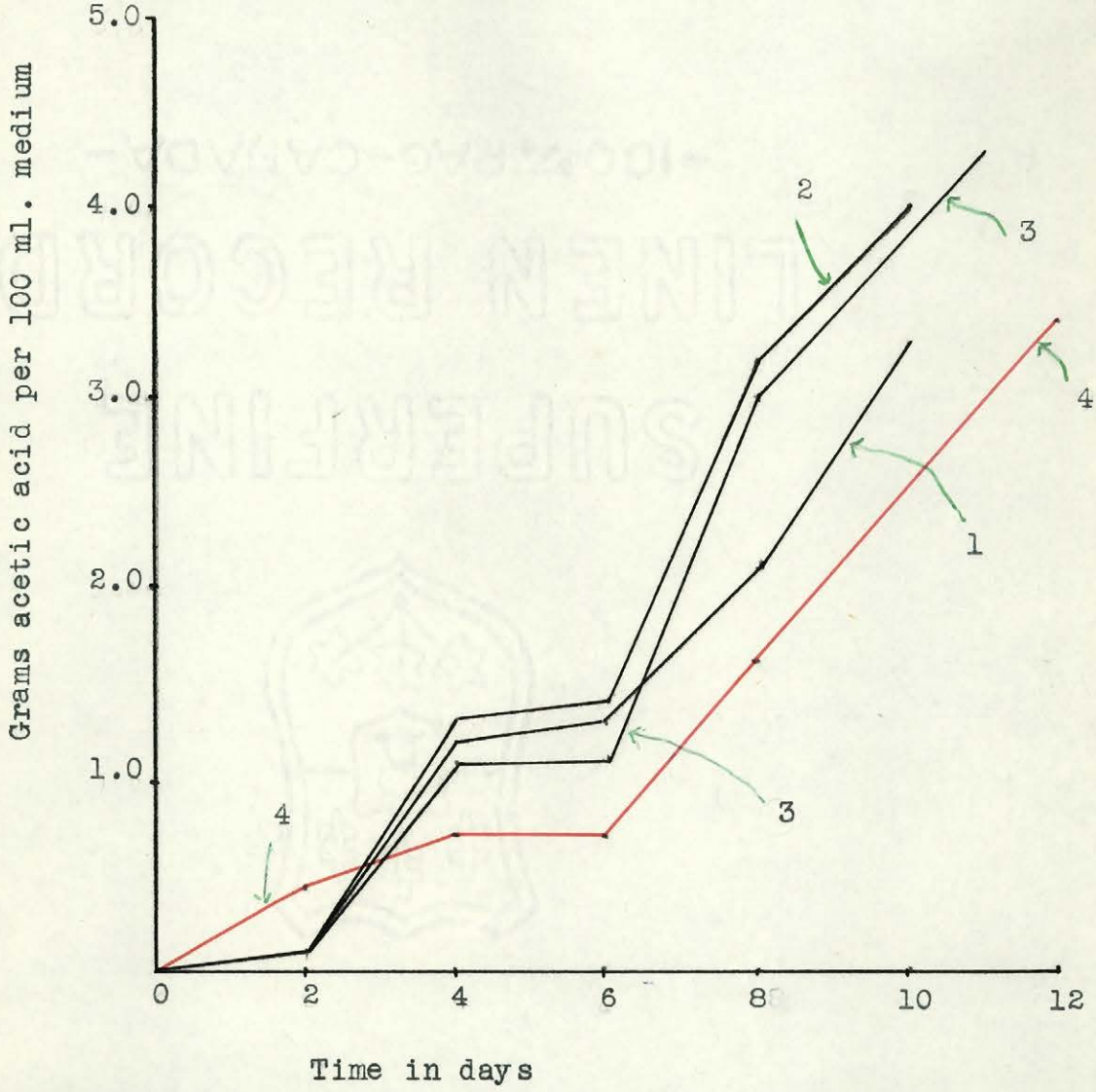


FIGURE 32A - THE EFFECT OF VARYING THE INITIAL pH OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER RANCENS

Curve 1 - pH 4.0
2 - pH 4.5
3 - pH 5.0
4 - pH 5.5

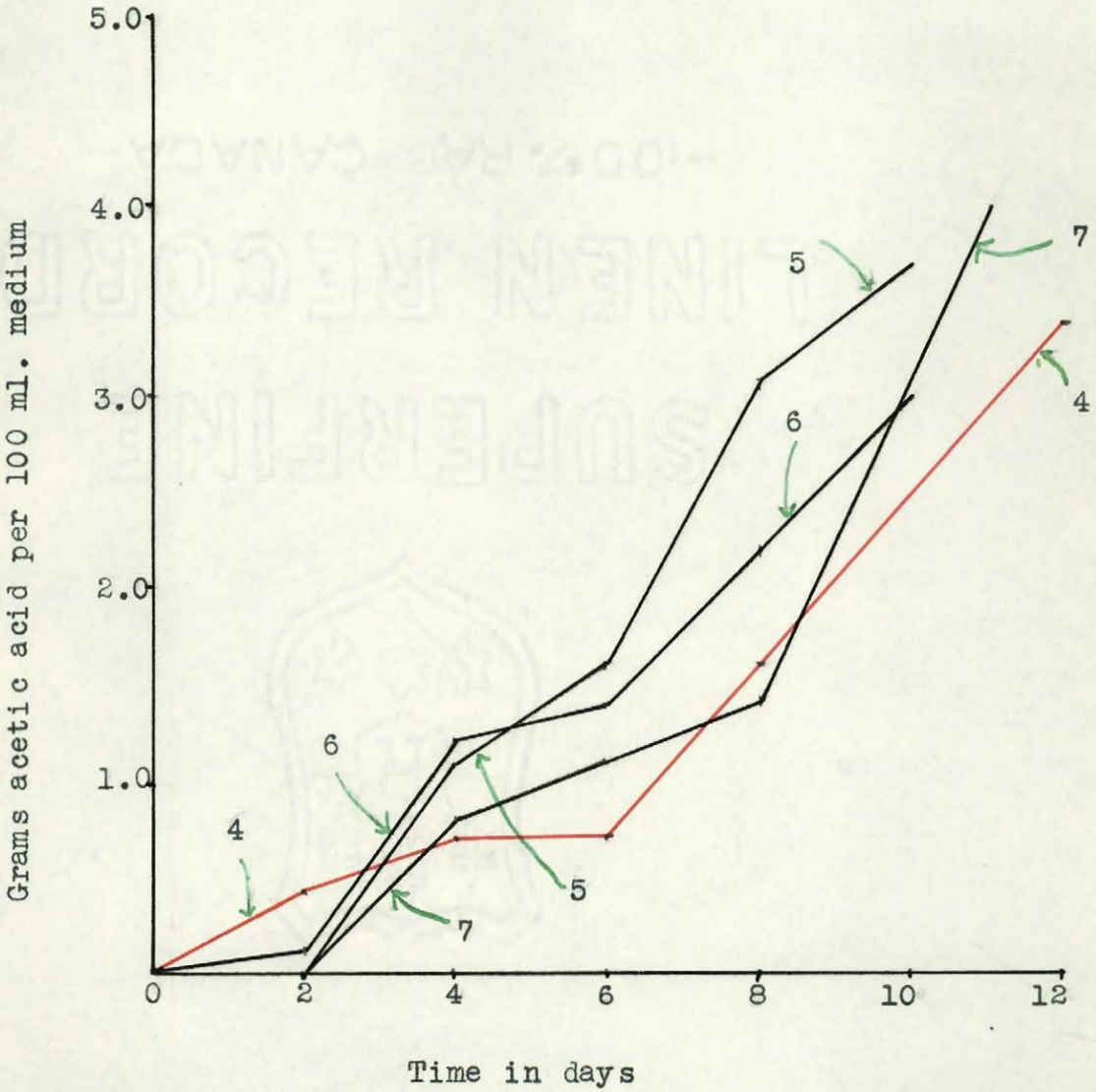


FIGURE 32B - THE EFFECT OF VARYING THE INITIAL pH OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER RANCENS

Curve 4 - pH 5.5
5 - pH 6.0
6 - pH 6.5
7 - pH 7.0

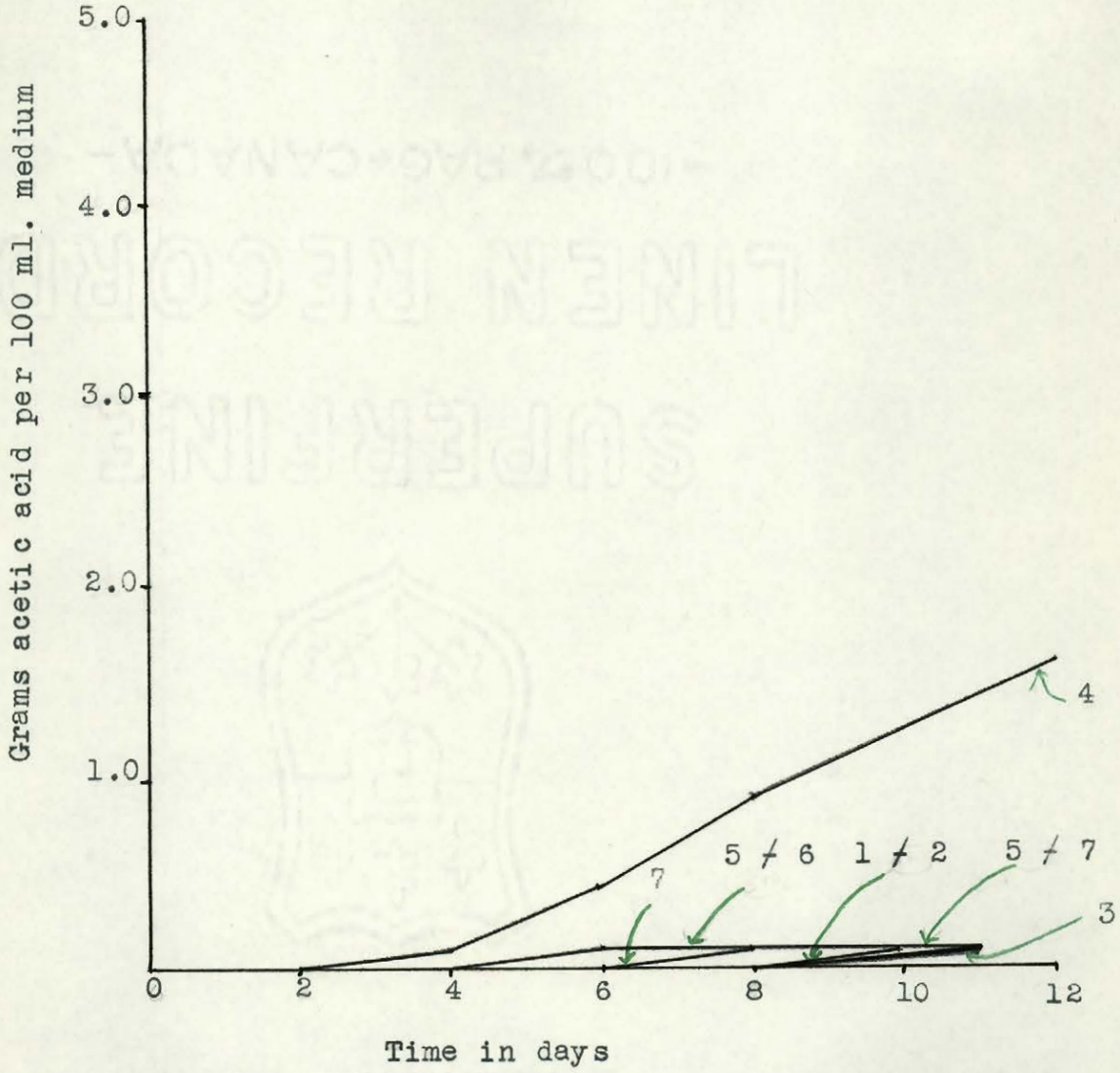


FIGURE 33 - THE EFFECT OF VARYING THE INITIAL pH OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER GLUCONICUM

Curve 1 - pH 4.0	Curve 5 - pH 6.0
2 - pH 4.5	6 - pH 6.5
3 - pH 5.0	7 - pH 7.0
4 - pH 5.5	

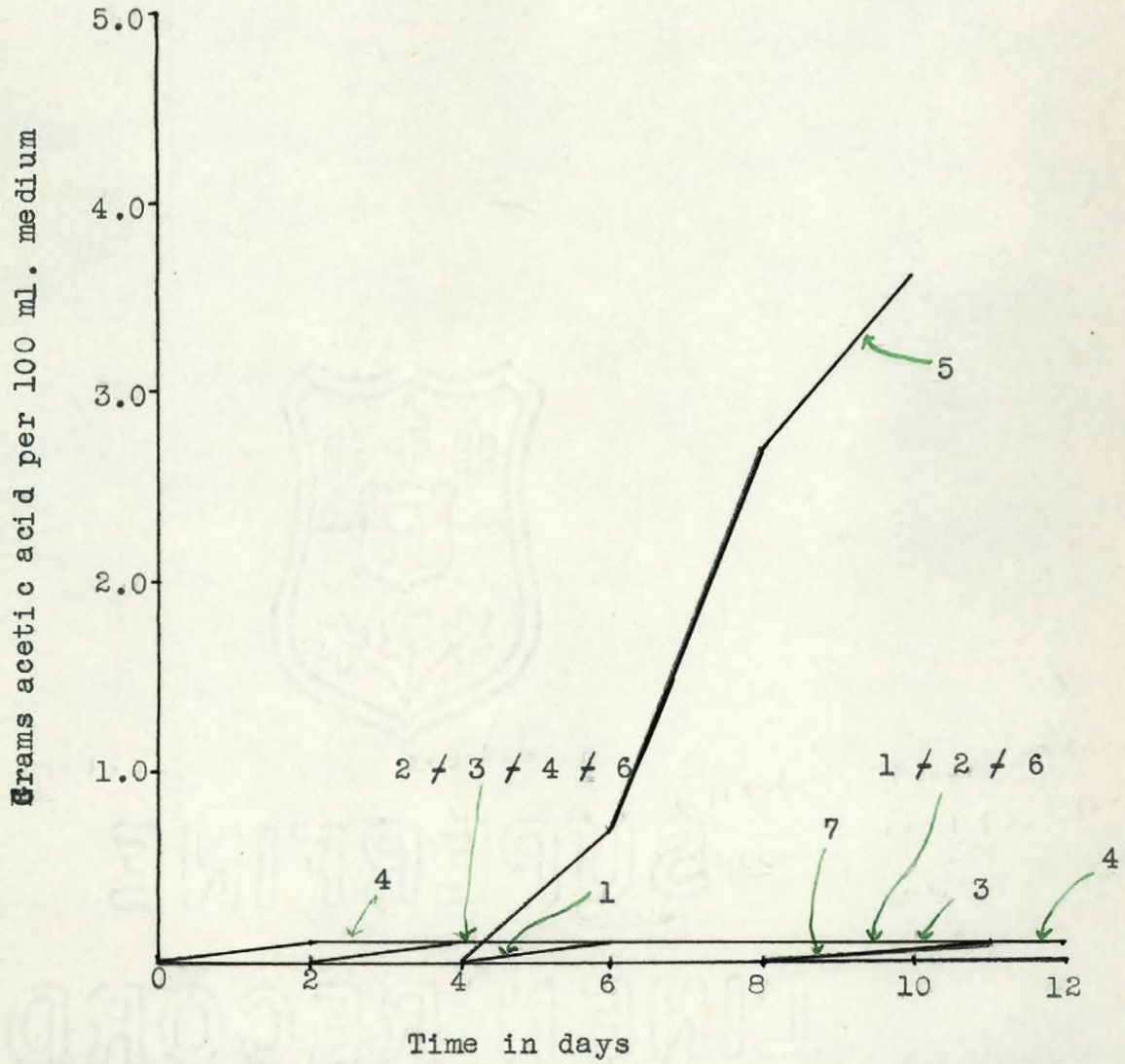


FIGURE 34 - THE EFFECT OF VARYING THE INITIAL pH OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER MELANOGENUM 1

Curve 1 - pH 4.0	Curve 5 - pH 6.0
2 - pH 4.5	6 - pH 6.5
3 - pH 5.0	7 - pH 7.0
4 - pH 5.5	

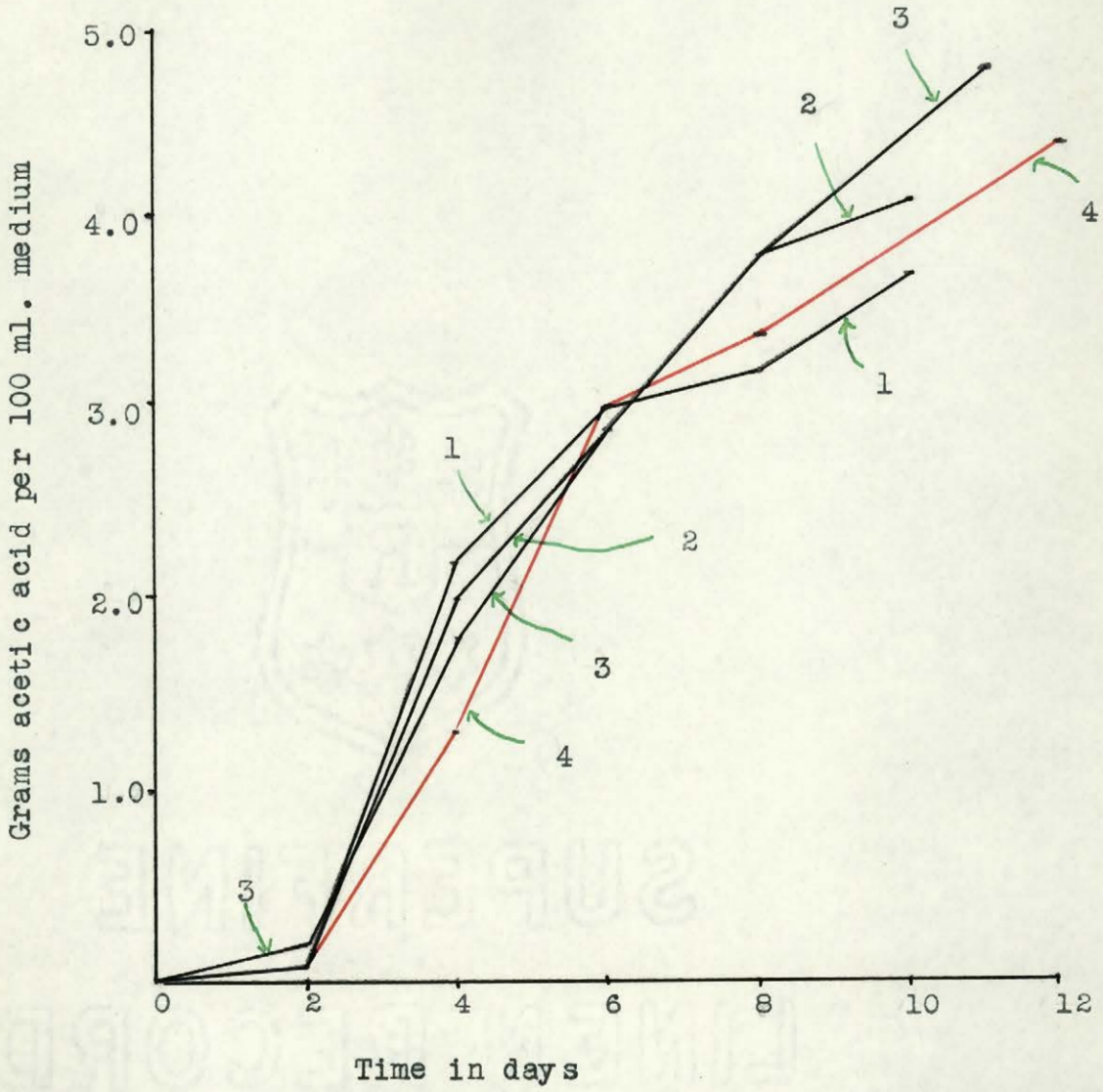


FIGURE 35A - THE EFFECT OF VARYING THE INITIAL pH OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER OXYDANS 1

Curve 1 - pH 4.0
2 - pH 4.5
3 - pH 5.0
4 - pH 5.5

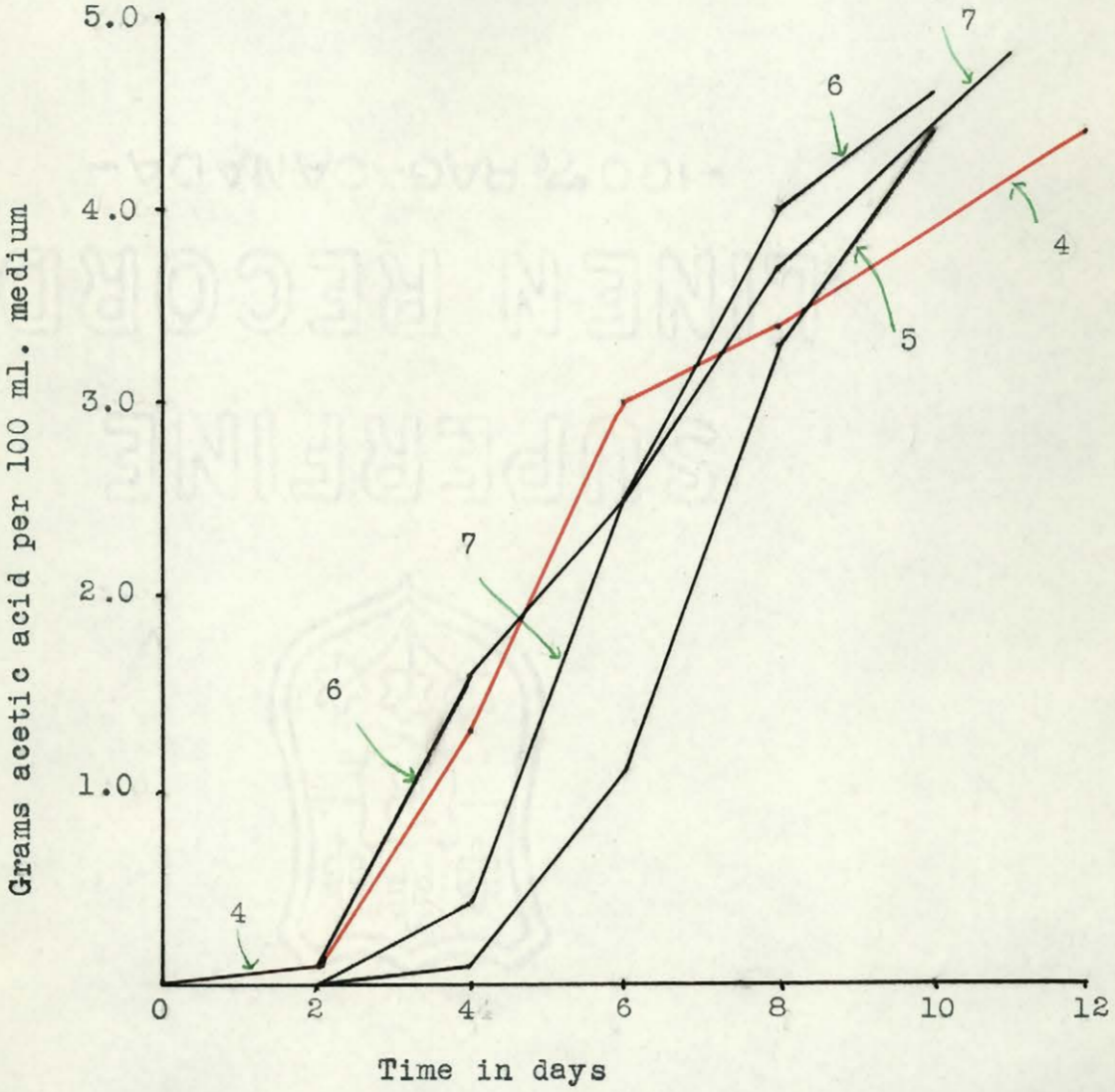


FIGURE 35B - THE EFFECT OF VARYING THE INITIAL pH OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER OXYDANS 1

Curve 4 - pH 5.5
5 - pH 6.0
6 - pH 6.5
7 - pH 7.0

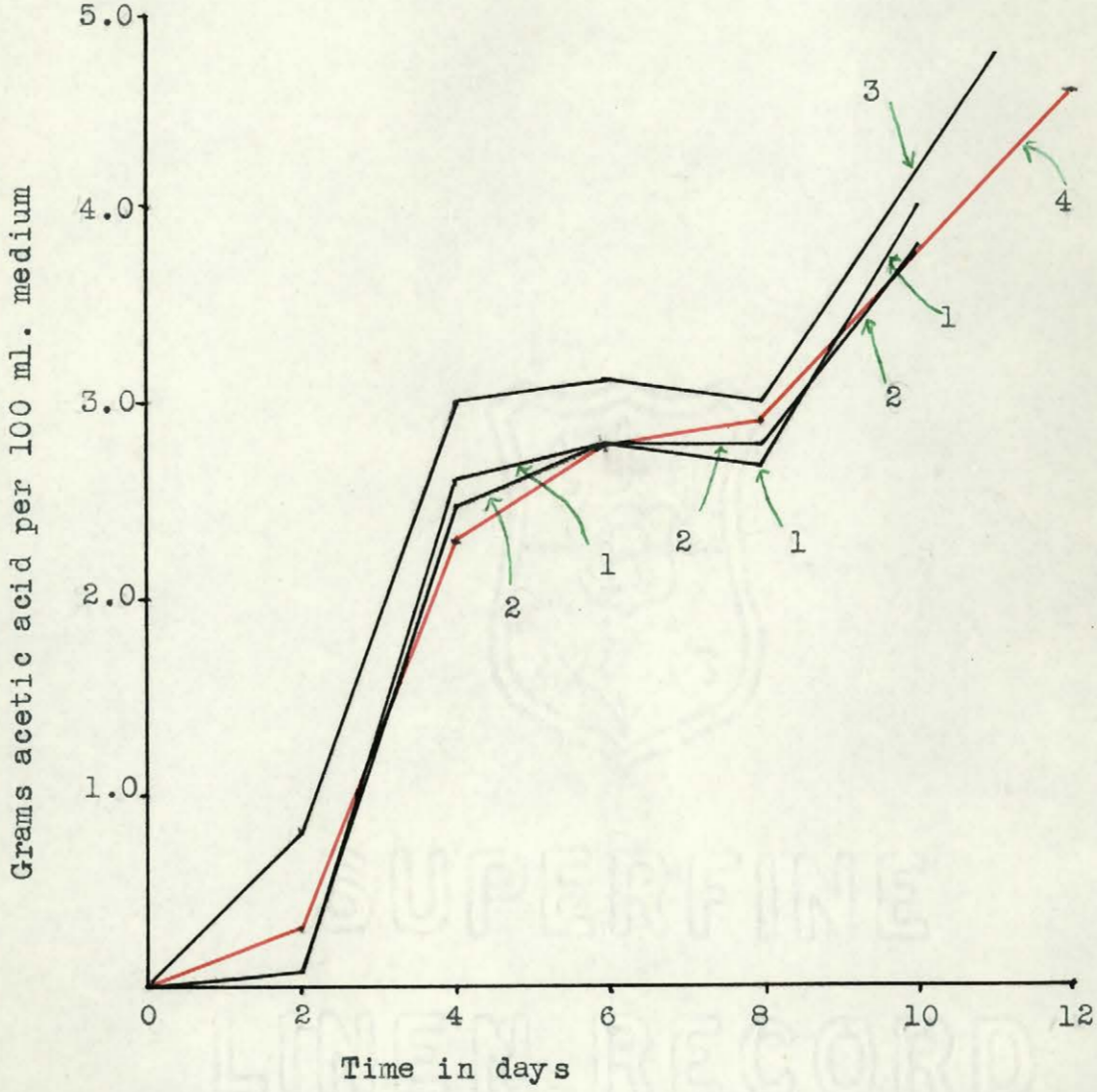


FIGURE 36A - THE EFFECT OF VARYING THE INITIAL pH OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER OXYDANS 2

Curve 1 - pH 4.0
2 - pH 4.5
3 - pH 5.0
4 - pH 5.5

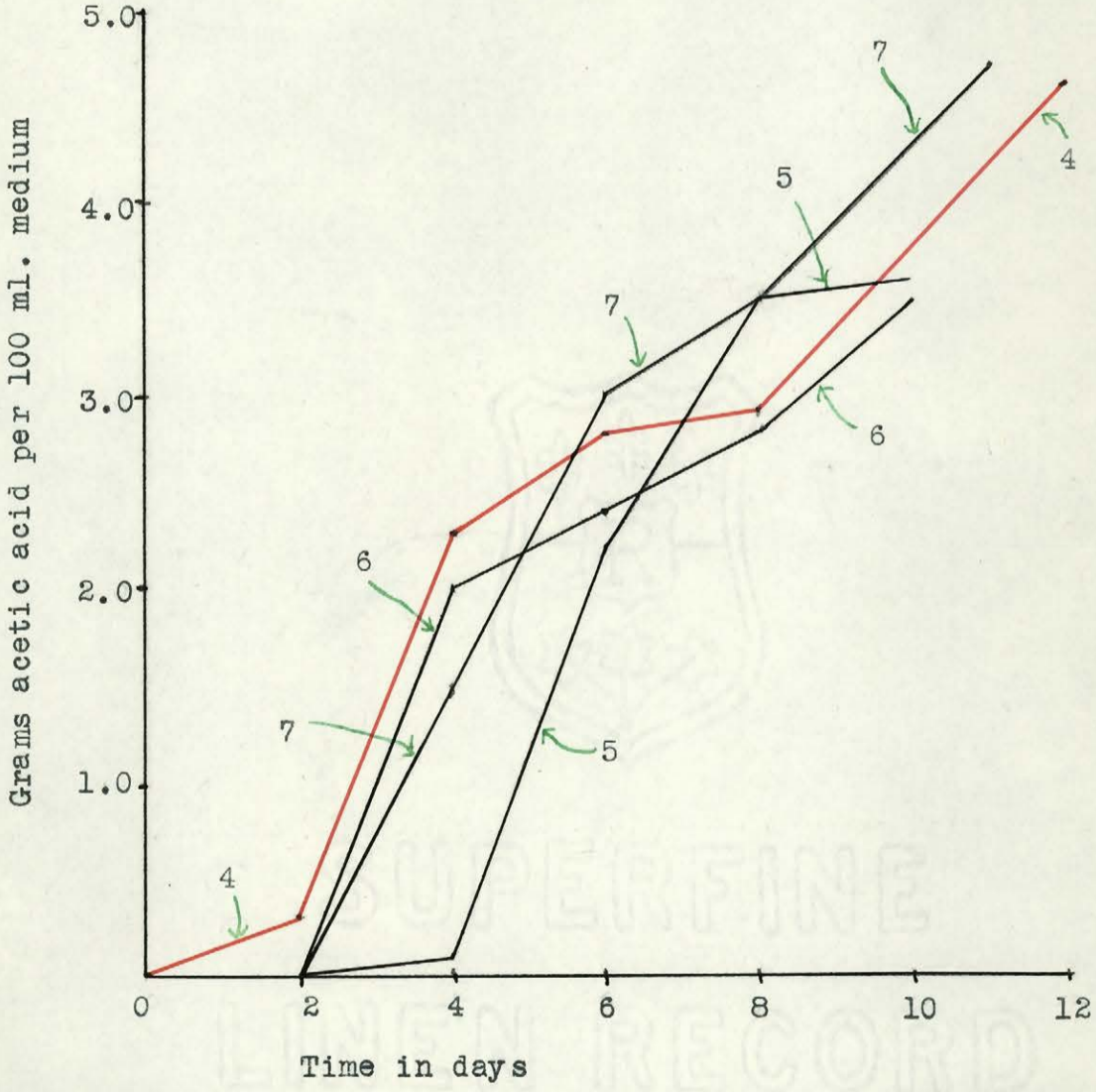


FIGURE 36B - THE EFFECT OF VARYING THE INITIAL pH OF THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER OXYDANS 2

Curve 4 - pH 5.5
5 - pH 6.0
6 - pH 6.5
7 - pH 7.0

the medium was between 6.0 and 7.0. Acetobacter kützingianum, Acetobacter aceti 1, Acetobacter oxydans 1, and Acetobacter oxydans 2 produced a lag in acid production when the medium was adjusted to pH 6.0; Acetobacter aceti 2 when the pH was adjusted to pH 7.0.

In terms of maximum acid production all organisms oxidized the alcohol. Acetobacter acetosum, Acetobacter ascendens, and Acetobacter aceti 1 each produced insignificantly different maximum amounts of acetic acid at all medium pH's studied. The other organisms produced maximum quantities of acetic acid that could not be classified in relation to the effect of the pH of the media used. The relationship was illogical even when the maximum eleven and twelve-day readings were replaced by the appropriate ten-day readings as read from the figures. Acetobacter aceti 2 did produce a smaller quantity of acid at pH's 4.0 to 4.5. In each instance a medium adjusted to pH 5.0 produced a good quantity of acid. The inability to relate the pH of the medium with acid production may be due to their spasmodic acid production. By referring to Table XXX on page 139 it is noticed that the organisms concerned, Acetobacter kützingianum, Acetobacter rancens, Acetobacter oxydans 1 and Acetobacter oxydans 2, are most spasmodic in acid production.

vi. The Size of Inocula in the Media

The results of the experiments are recorded in Figures 37 to 46 (pp.192-201), and Appendix Tables XXIII to XXVI (pp. 267-270).

Acetobacter gluconicum and Acetobacter melanogenum 1 produced insignificant amounts of acetic acid when inocula three and five times the standard size were used. As stated in the experimental results of the effect of the pH of the medium, both species produced a good acetic acid yield when standard sized inocula were used.

Acetobacter kützingianum, Acetobacter aceti 2, Acetobacter rancens, Acetobacter oxydans 1 and Acetobacter oxydans 2 produced a decided lag in acetic acid production when three and five times the standard sized inocula were used, and the amount of acid produced in ten days never reached the quantity produced by the smallest inoculum. Acetobacter aceti 1 produced a somewhat similar effect. The rates of acid production when inocula were three and five times the standard size were in better agreement than when inocula were of standard size. Acetobacter acetosum and Acetobacter ascendens each showed more agreement of the rate of acid production with different sized inocula.

In terms of the variation giving a maximum yield of acetic acid, each organism oxidized alcohol satisfactorily when standard sized inocula were used. Acetobacter acetosum and Acetobacter aceti 1 each produced insignificantly different maximum amounts of acetic acid when standard sized, three and five times standard

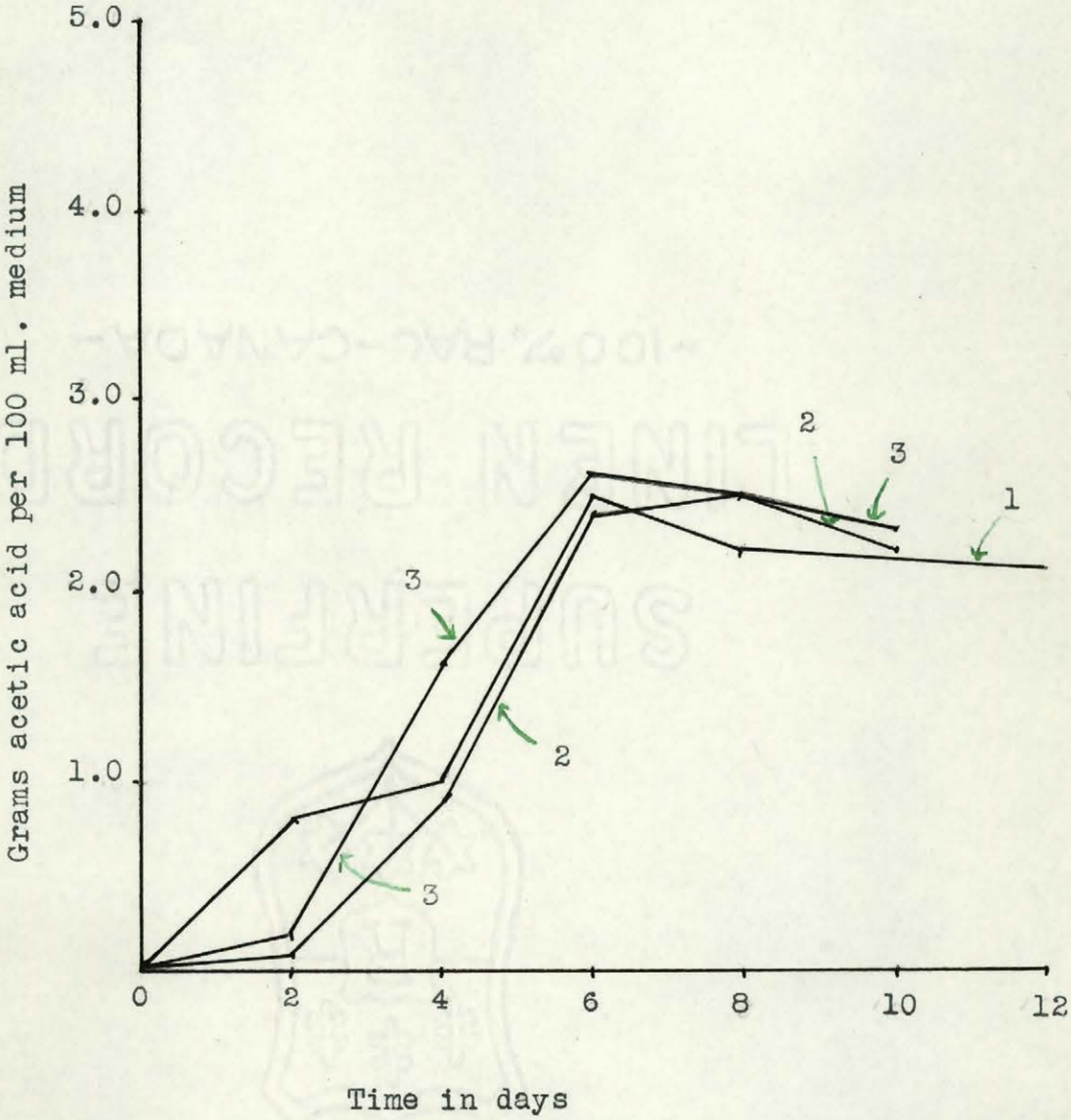


FIGURE 37 - THE EFFECT OF VARYING THE SIZE OF THE ACETOBACTER ACETOSUM INOCULUM IN THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED

Curve 1 - Inoculum, standard
2 - Inoculum, 3 x standard
3 - Inoculum, 5 x standard

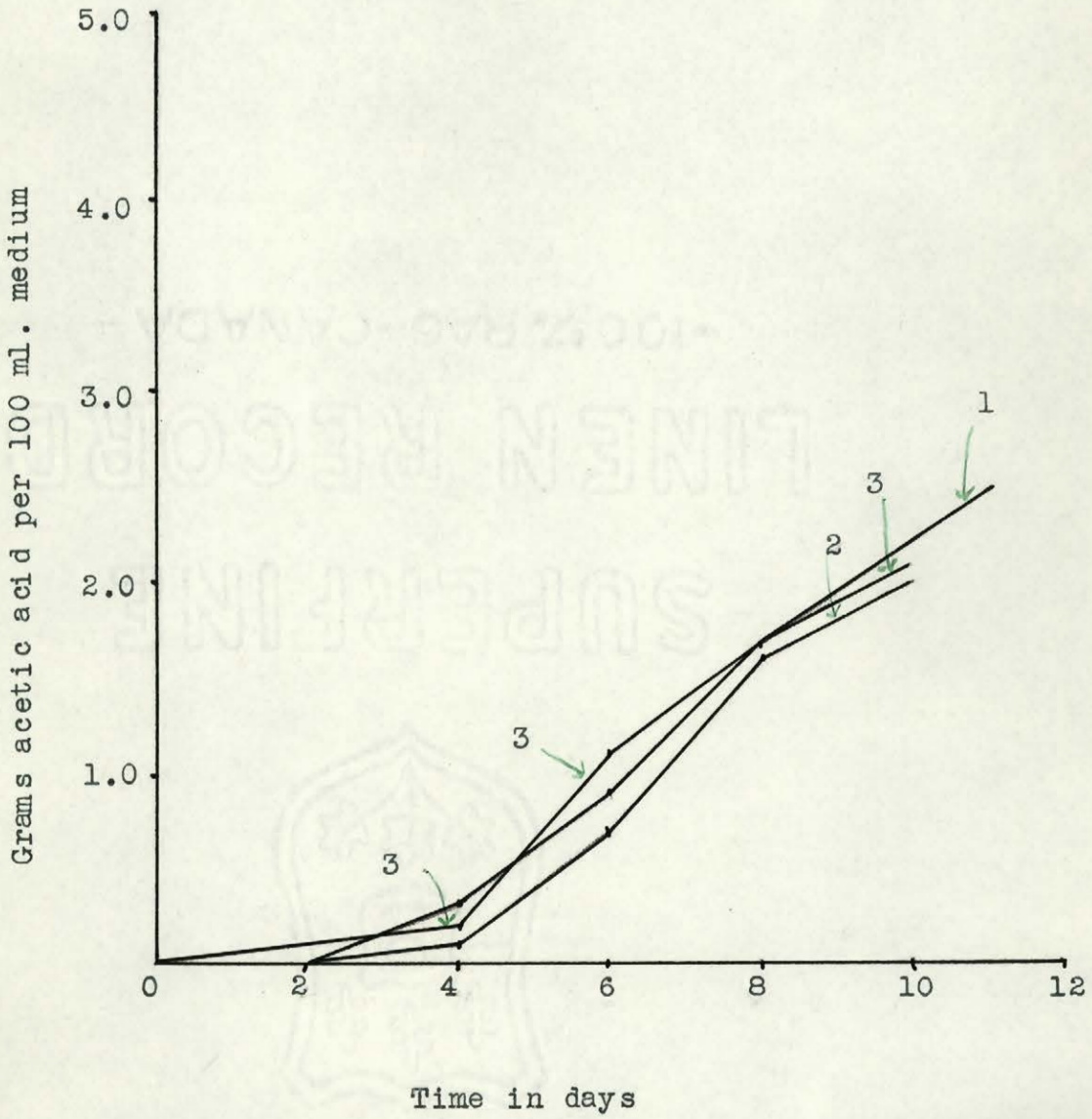


FIGURE 38 - THE EFFECT OF VARYING THE SIZE OF THE ACETOBACTER ASCENDENS INOCULUM IN THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED

Curve 1 - Inoculum, standard
2 - Inoculum, 3 x standard
3 - Inoculum, 5 x standard

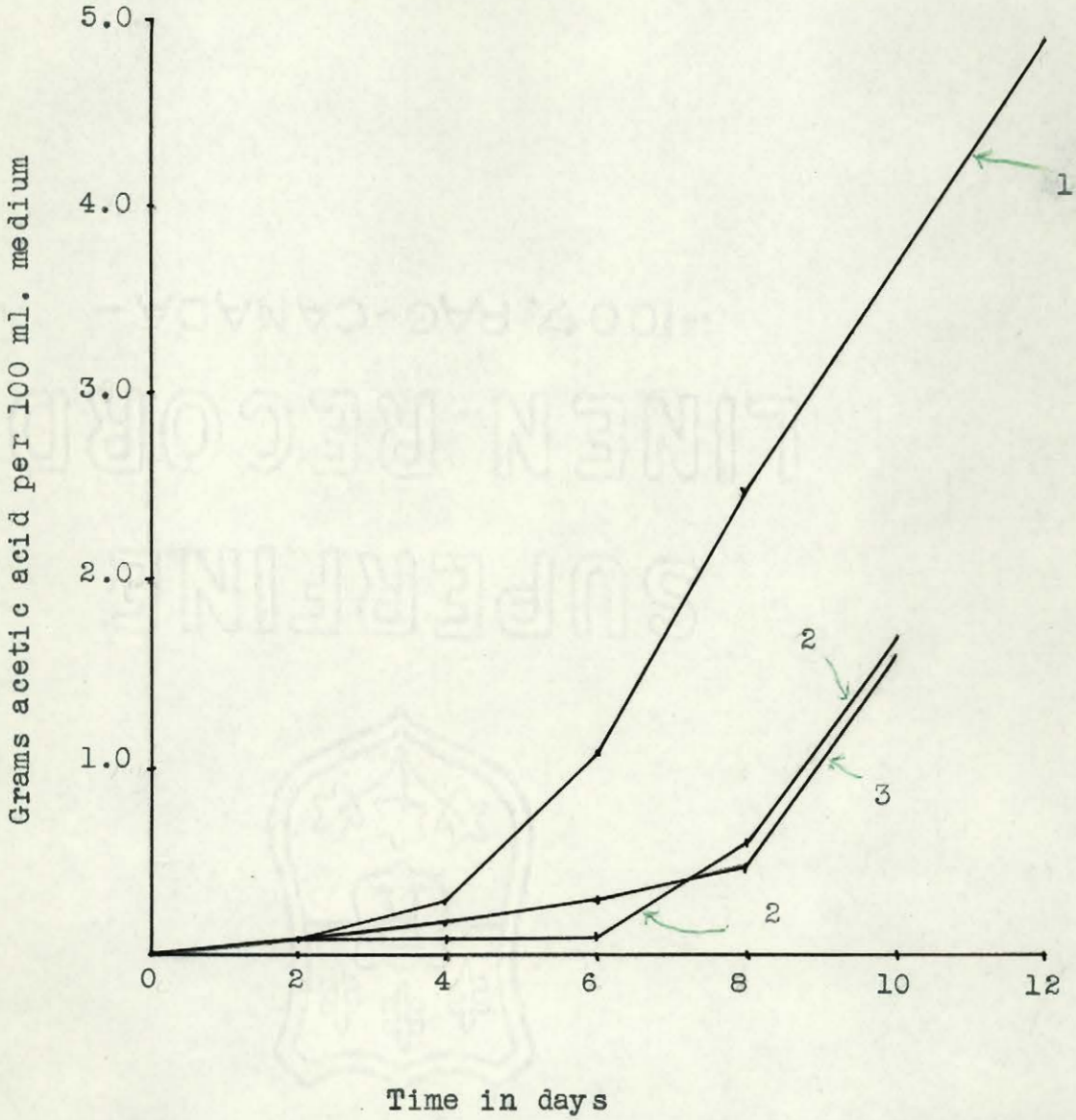


FIGURE 39 - THE EFFECT OF VARYING THE SIZE OF THE ACETOBACTER KÜTZINGIANUM INOCULUM IN THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED

Curve 1 - Inoculum, standard
2 - Inoculum, 3 x standard
3 - Inoculum, 5 x standard

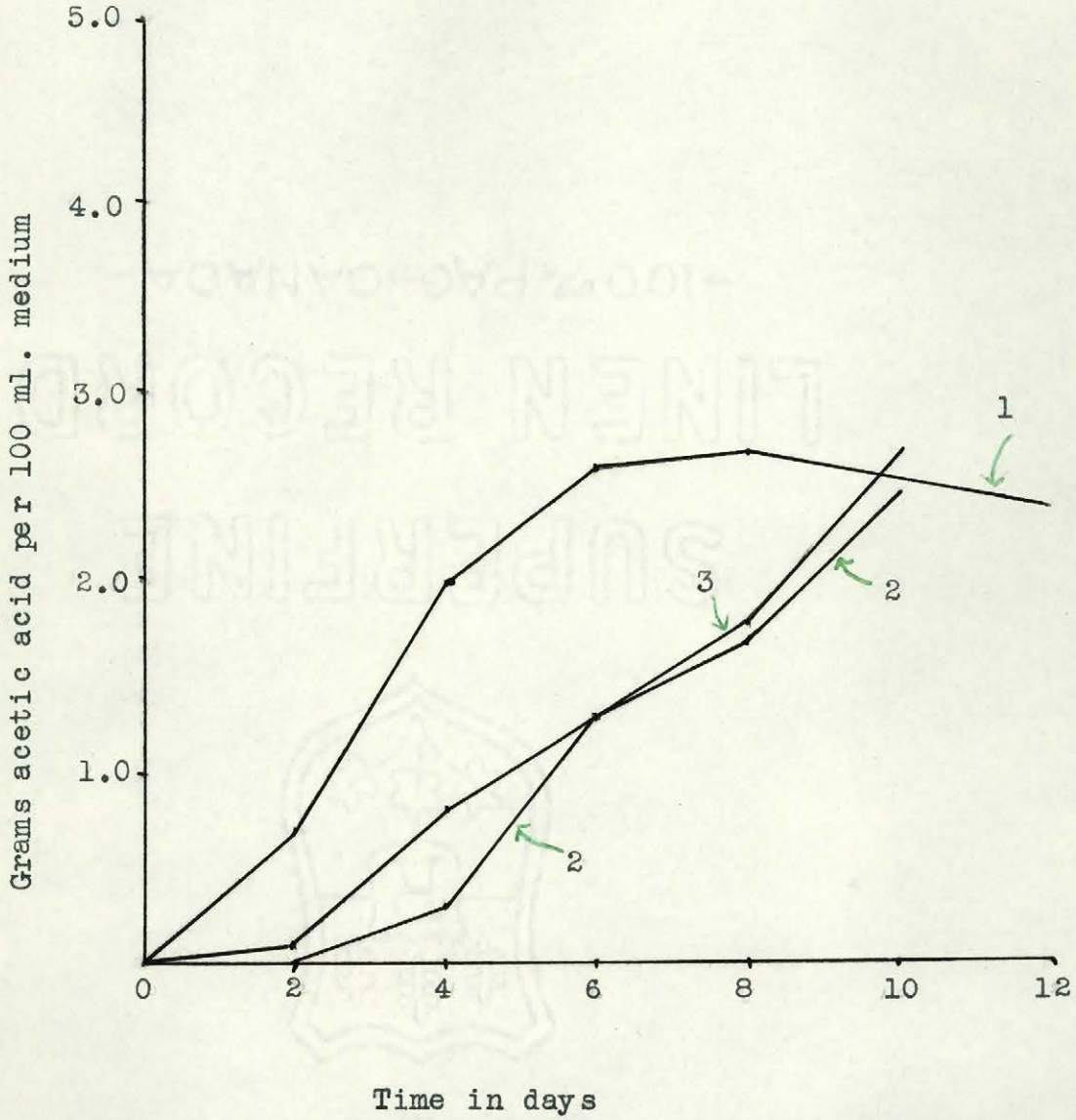


FIGURE 40 - THE EFFECT OF VARYING THE SIZE OF THE ACETOBACTER ACETI 1 INOCULUM IN THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED

Curve 1 - Inoculum, standard
2 - Inoculum, 3 x standard
3 - Inoculum, 5 x standard

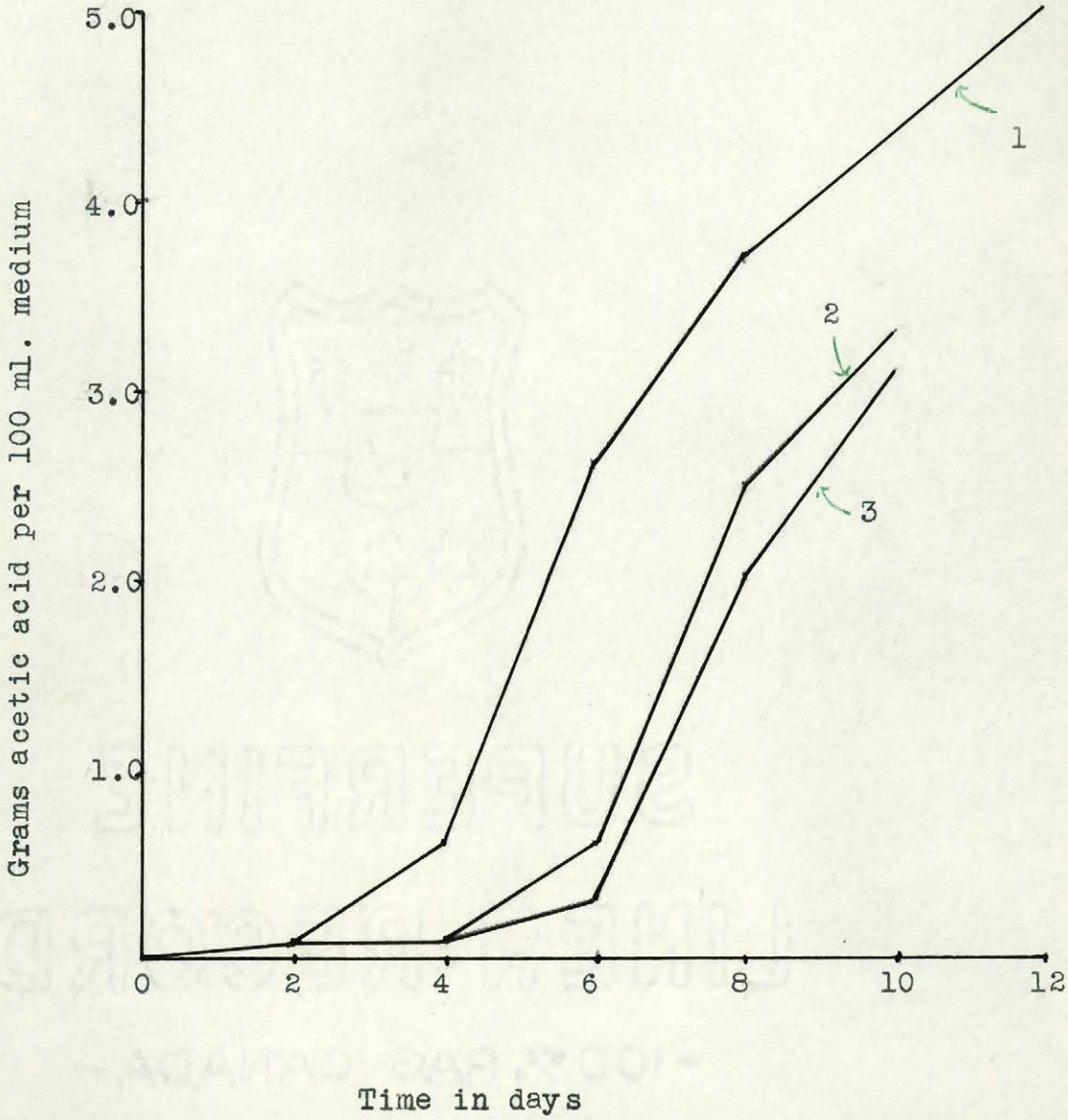


FIGURE 41 - THE EFFECT OF VARYING THE SIZE OF THE ACETOBACTER ACETI 2 INOCULUM IN THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED

Curve 1 - Inoculum, standard
2 - Inoculum, 3 x standard
3 - Inoculum, 5 x standard

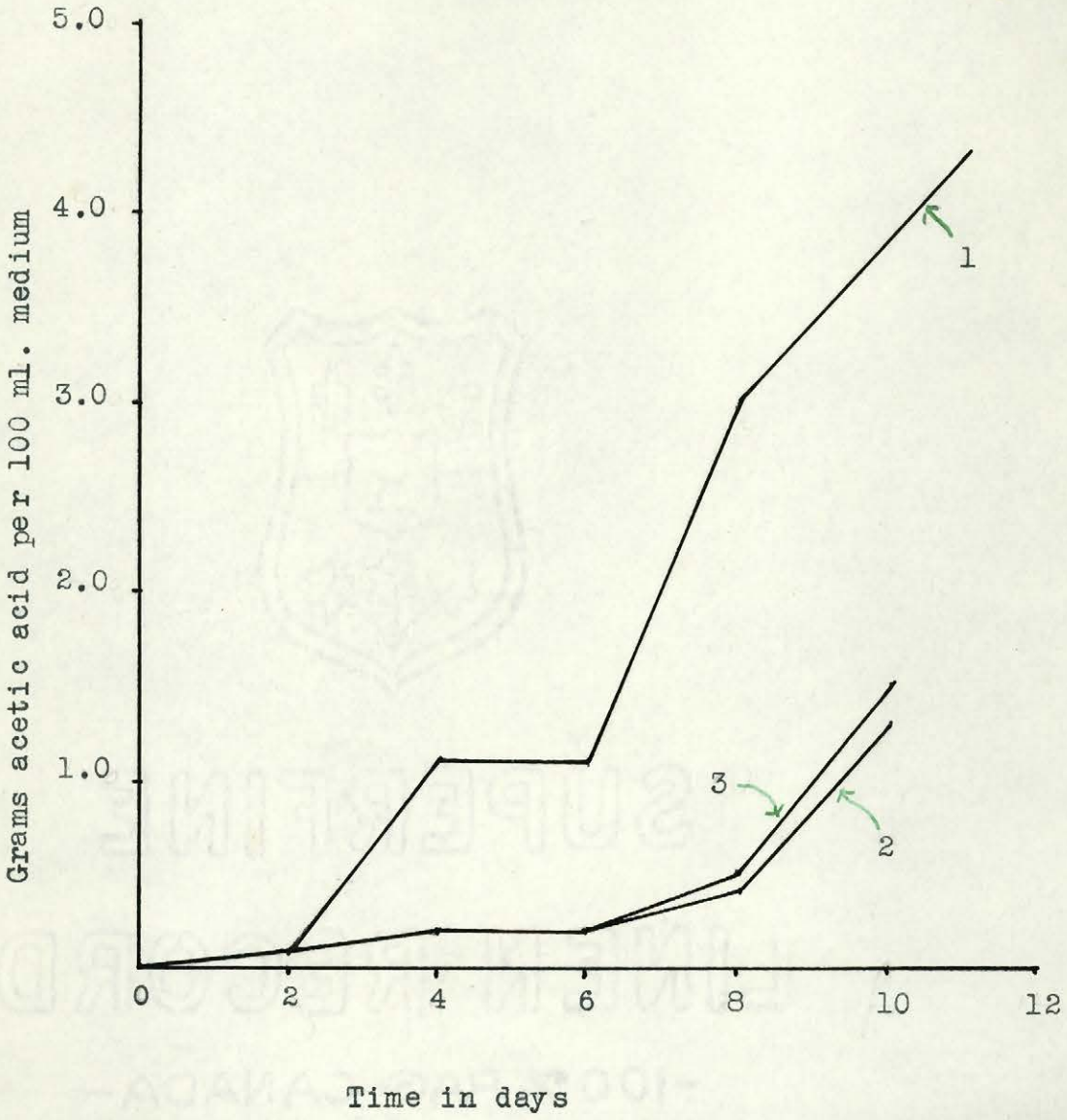


FIGURE 42 - THE EFFECT OF VARYING THE SIZE OF THE ACETOBACTER RANCENS INOCULUM IN THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED

Curve 1 - Inoculum, standard
2 - Inoculum, 3 x standard
3 - Inoculum, 5 x standard

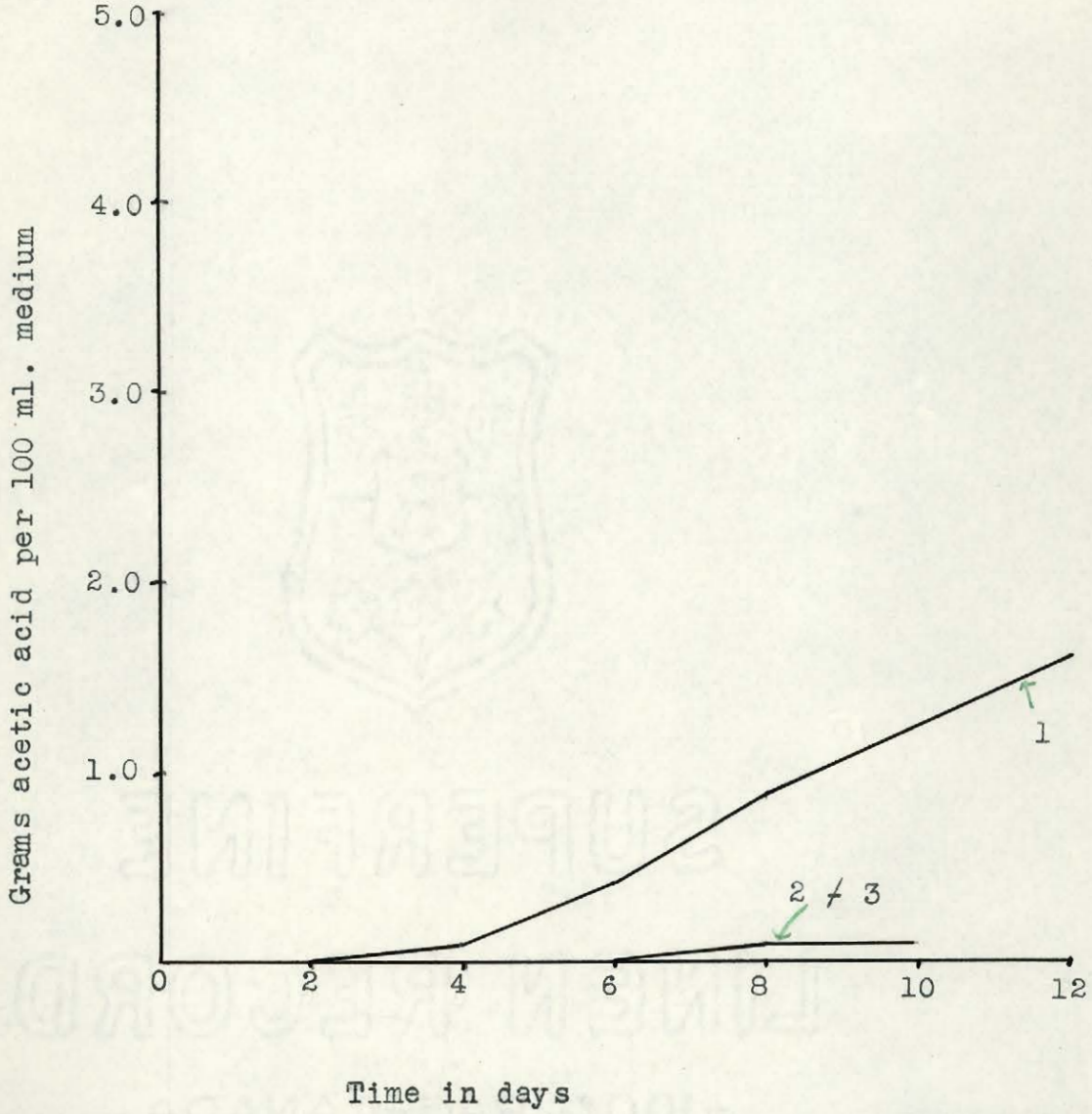


FIGURE 43 - THE EFFECT OF VARYING THE SIZE OF THE ACETOBACTER GLUCONICUM INOCULUM IN THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED

Curve 1 - Inoculum, standard
2 - Inoculum, 3 x standard
3 - Inoculum, 5 x standard

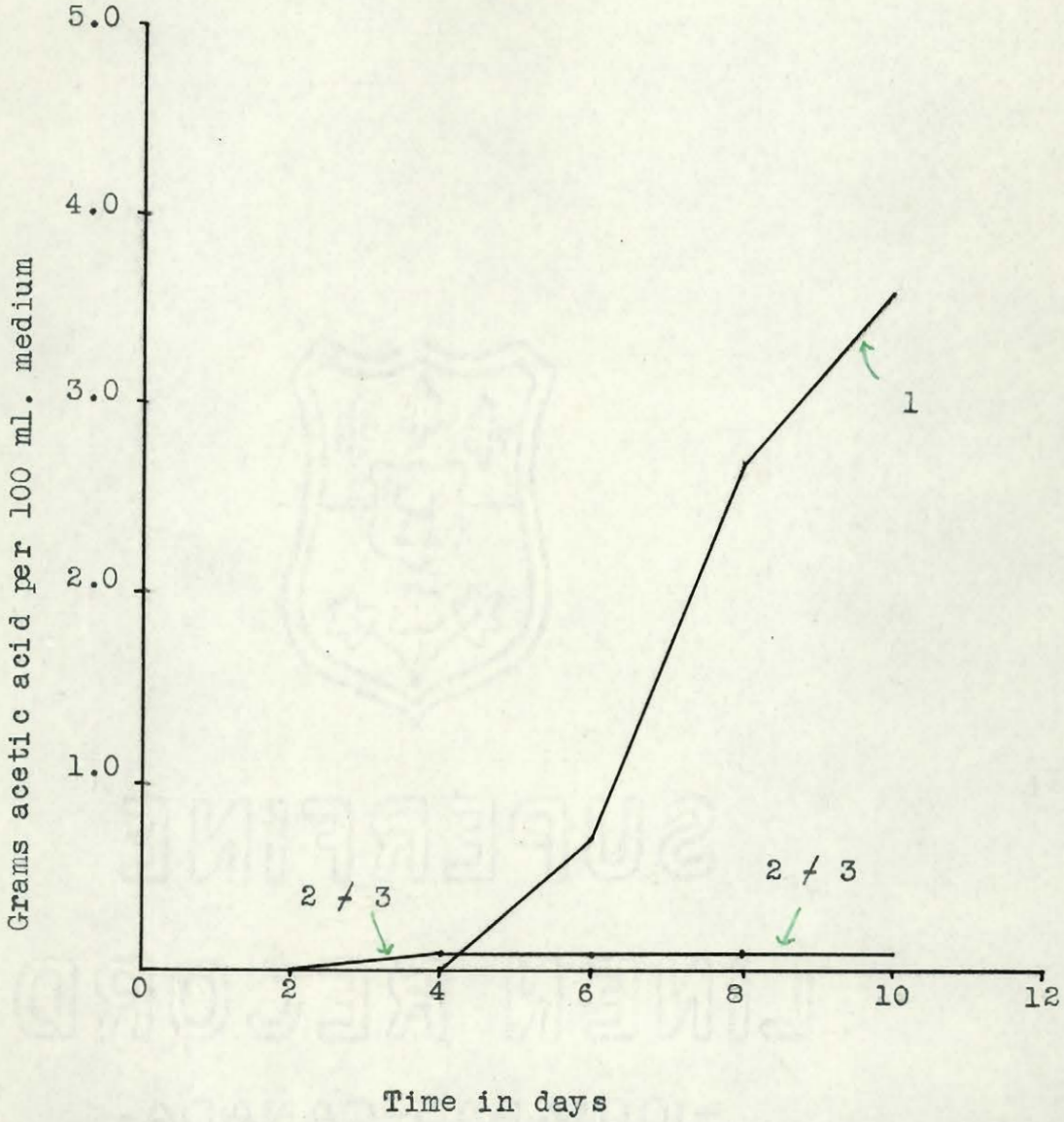


FIGURE 44 - THE EFFECT OF VARYING THE SIZE OF THE ACETOBACTER MELANOGENUM 1 INOCULUM IN THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED

Curve 1 - Inoculum, standard
2 - Inoculum, 3 x standard
3 - Inoculum, 5 x standard

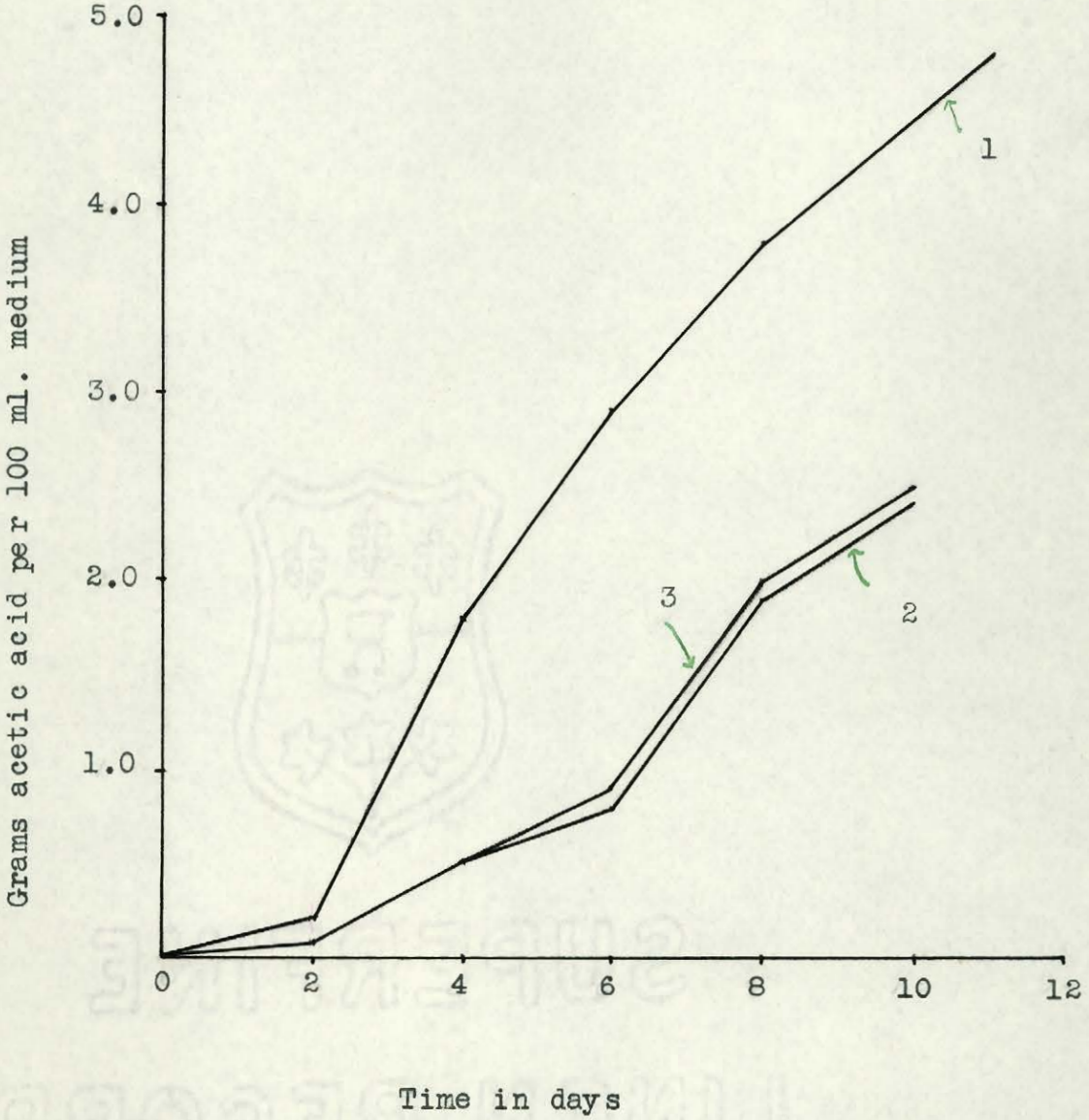


FIGURE 45 - THE EFFECT OF VARYING THE SIZE OF THE ACETOBACTER OXYDANS 1 INOCULUM IN THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED

Curve 1 - Inoculum, standard
2 - Inoculum, 3 x standard
3 - Inoculum, 5 x standard

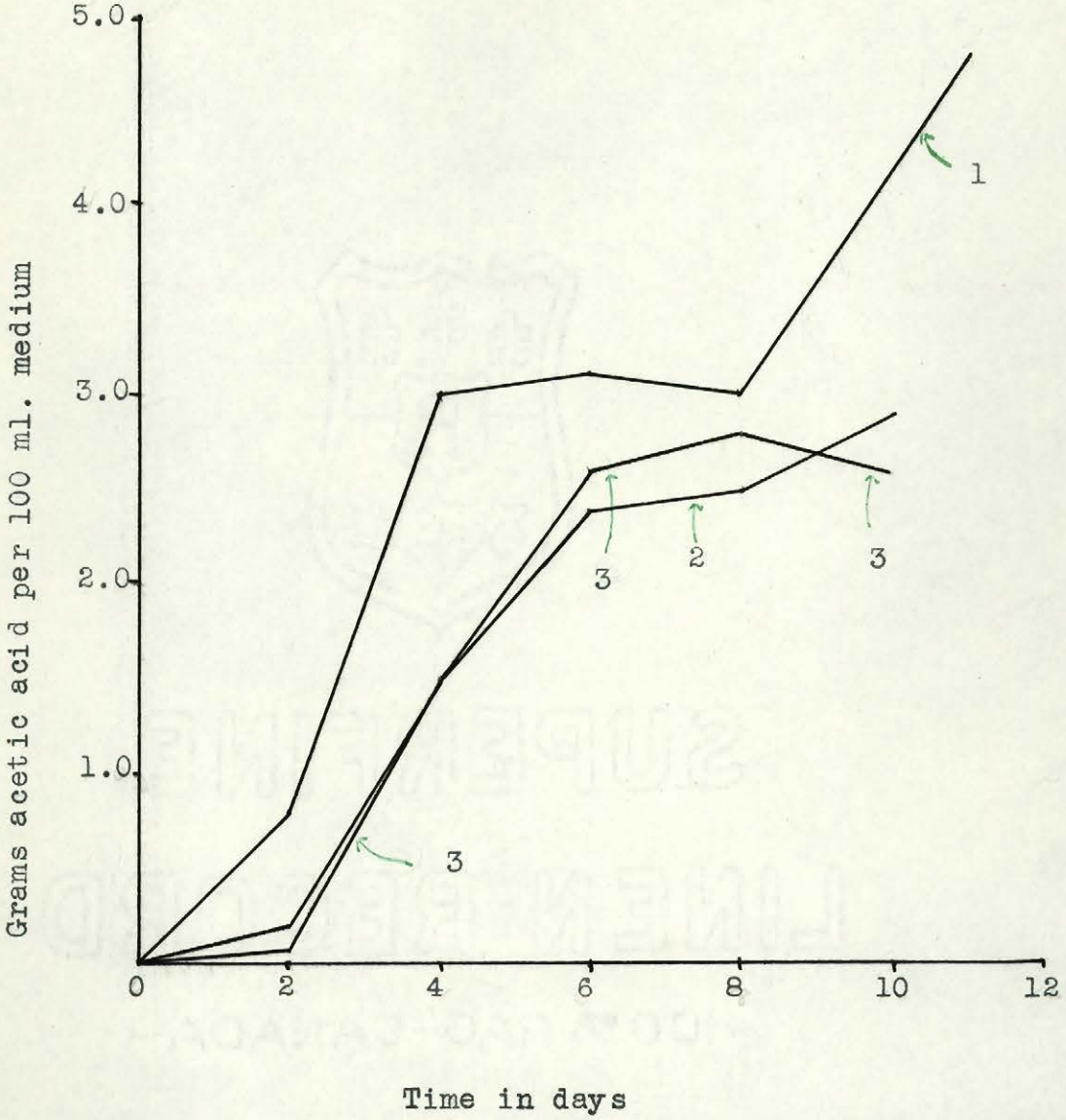


FIGURE 46 - THE EFFECT OF VARYING THE SIZE OF THE ACETOBACTER OXYDANS 2 INOCULUM IN THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED

Curve 1 - Inoculum, standard
2 - Inoculum, 3 x standard
3 - Inoculum, 5 x standard

sized inocula were used; Acetobacter ascendens when standard and five times standard sized inocula were used.

vii. Mixed Inocula in the Media

The results of the experiments are recorded in Figures 47 to 59 (pp.203-215), and Appendix Tables XXVII to XXX (pp.271 - 274) and Appendix Table XXIII (p.267).

A mixture of each organism with Acetobacter aceti 1:

Acetobacter aceti 1 produced an insignificant amount of acetic acid in media containing 6 and 9 per cent ethyl alcohol, but produced good quantities at a good rate in media containing 3 per cent. For the experiment media containing the per cent of ethyl alcohol that produced the maximum amount of acetic acid with each of the other organisms were used. Therefore it seemed best for discussion of acetic acid production by mixed inocula to separate the types of mixtures into groups determined by the initial concentration of ethyl alcohol in the fermenting media. A comparison of the rate and amount of acetic acid produced by the mixture could then be made with that of the individual organisms, as noted in appropriate previous experiments.

A medium containing 9 per cent ethyl alcohol was inoculated with a mixture of Acetobacter turbidans and Acetobacter aceti 1. The mixed inoculum produced an insignificant maximum amount of acetic acid. The individual organisms also produced an insignificant amount of acid.

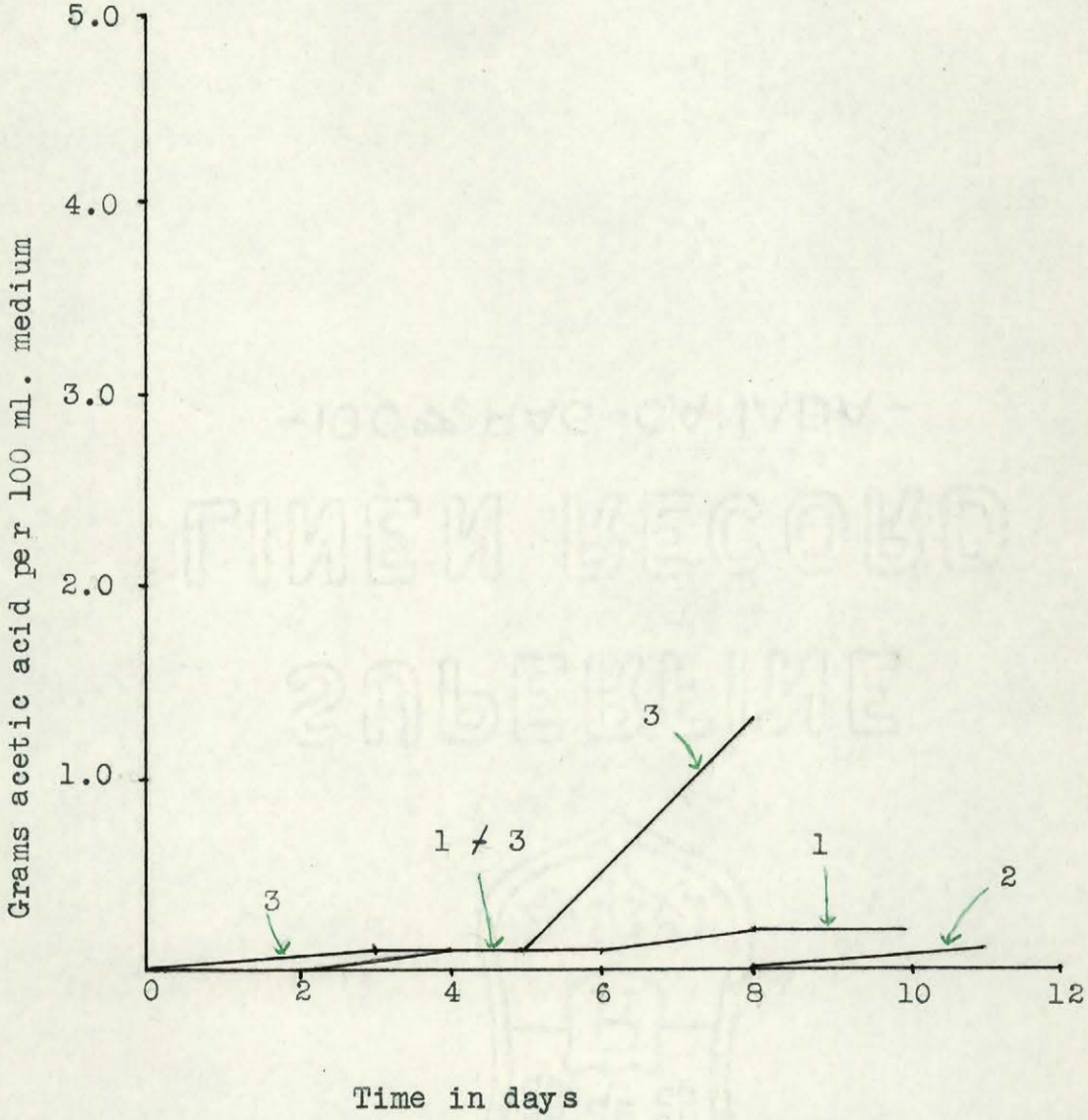


FIGURE 47 - THE EFFECT OF MIXED INOCULA IN THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER SUBOXYDANS

Curve 1 - Mixture, A. suboxydans ≠ A. aceti 1
2 - A. suboxydans
3 - A. aceti 1

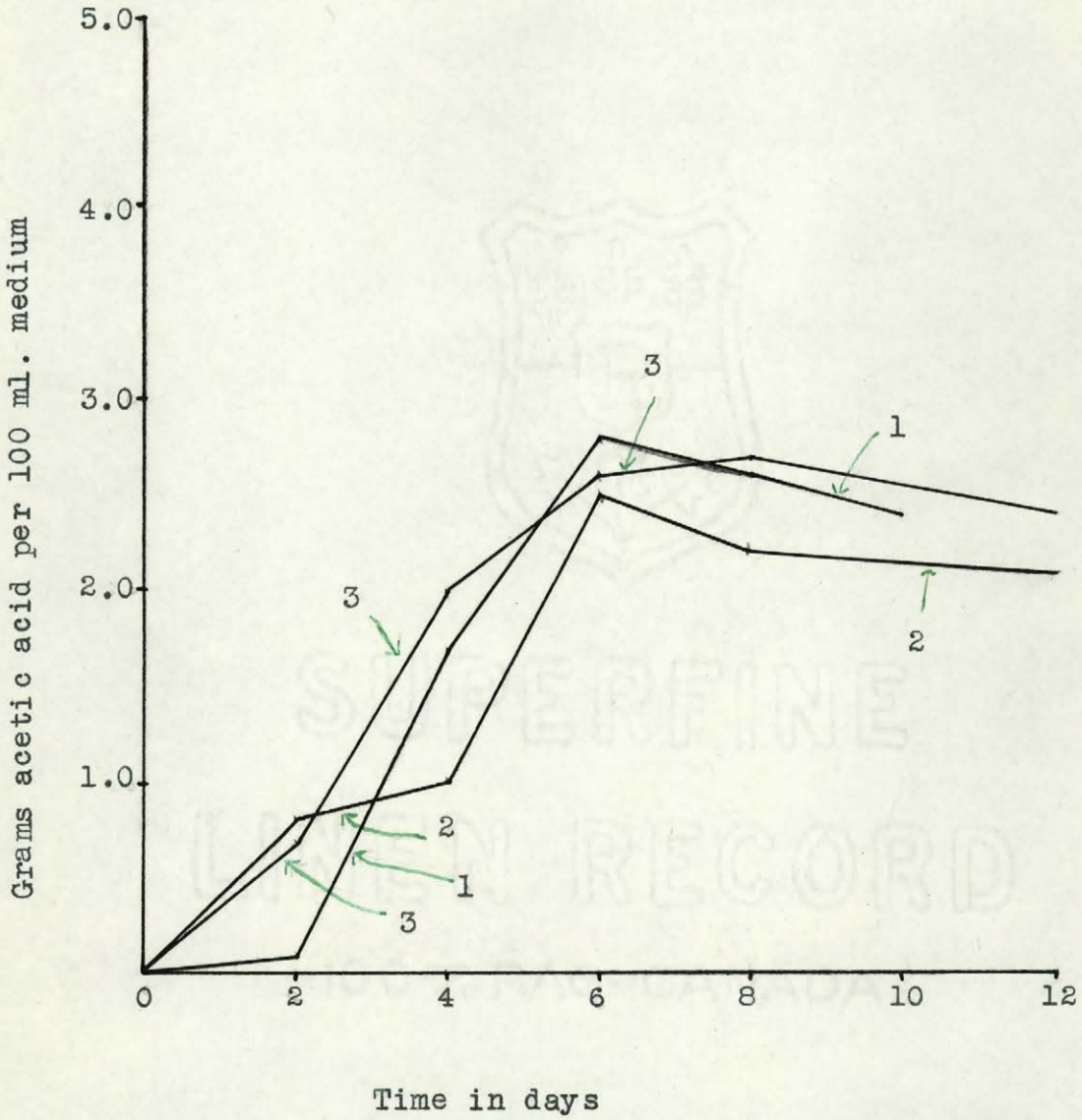


FIGURE 48 - THE EFFECT OF MIXED INOCULA IN THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED - ACETOBACTER ACETOSUM

Curve 1 - Mixture, A. acetosum + A. aceti 1
2 - A. acetosum
3 - A. aceti 1

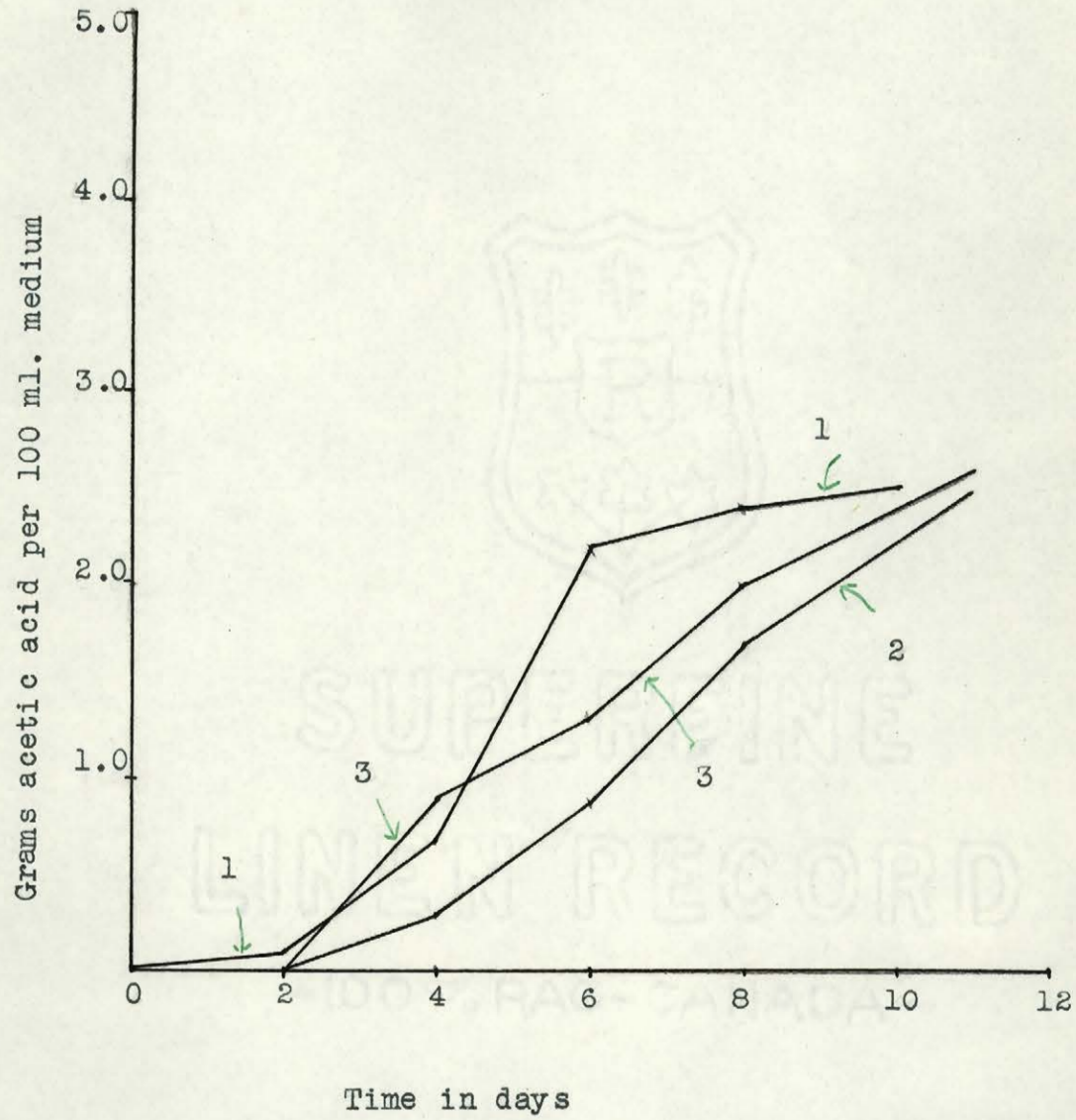


FIGURE 49 - THE EFFECT OF MIXED INOCULA IN THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED - ACETOBACTER ASCENDENS

Curve 1 - Mixture, A. ascendens + A. aceti 1
2 - A. ascendens
3 - A. aceti 1

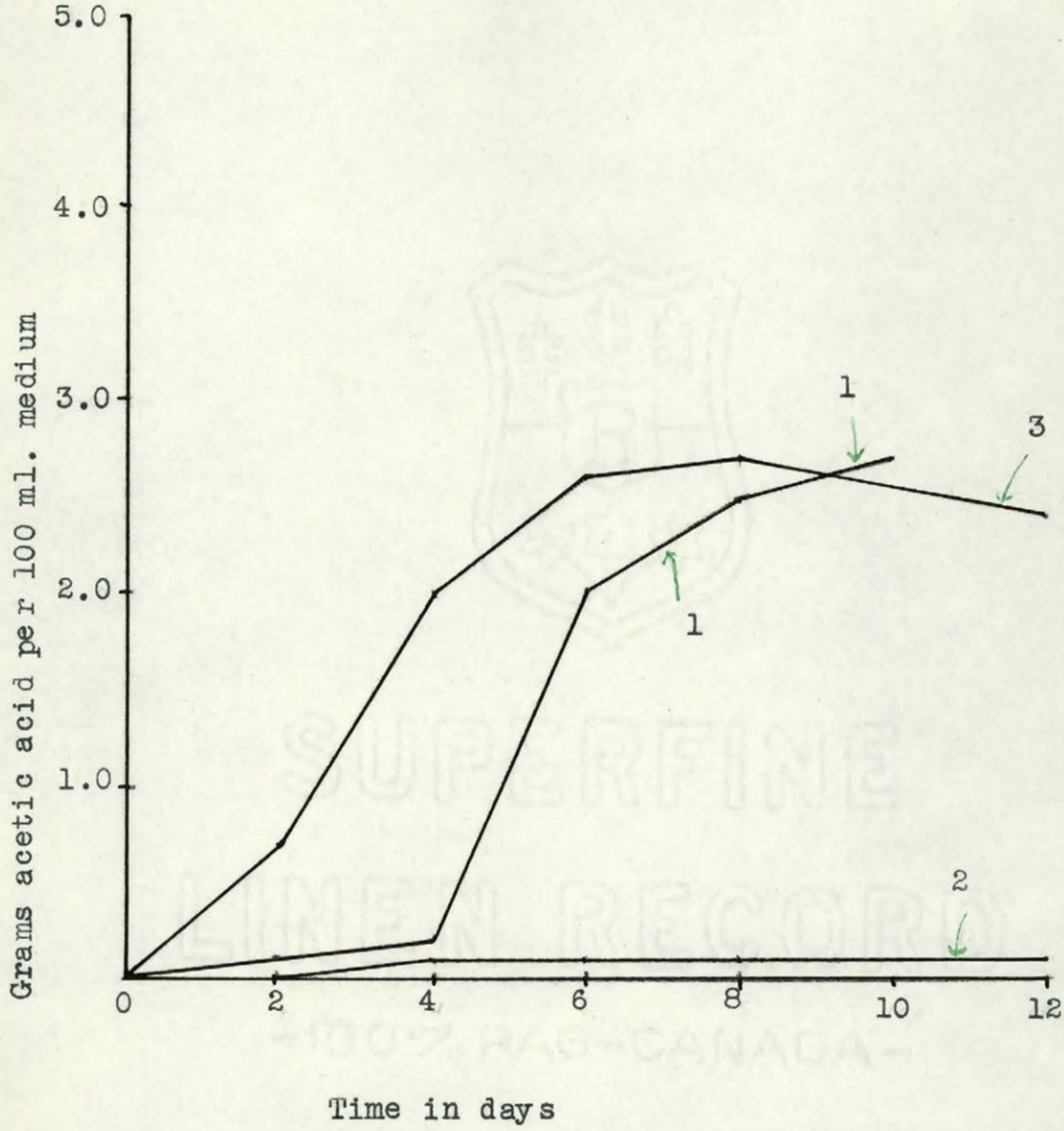


FIGURE 50 - THE EFFECT OF MIXED INOCULA IN THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED - ACETOBACTER PASTEURIANUM

Curve 1 - Mixture, A. pasteurianum + A. aceti 1
2 - A. pasteurianum
3 - A. aceti 1

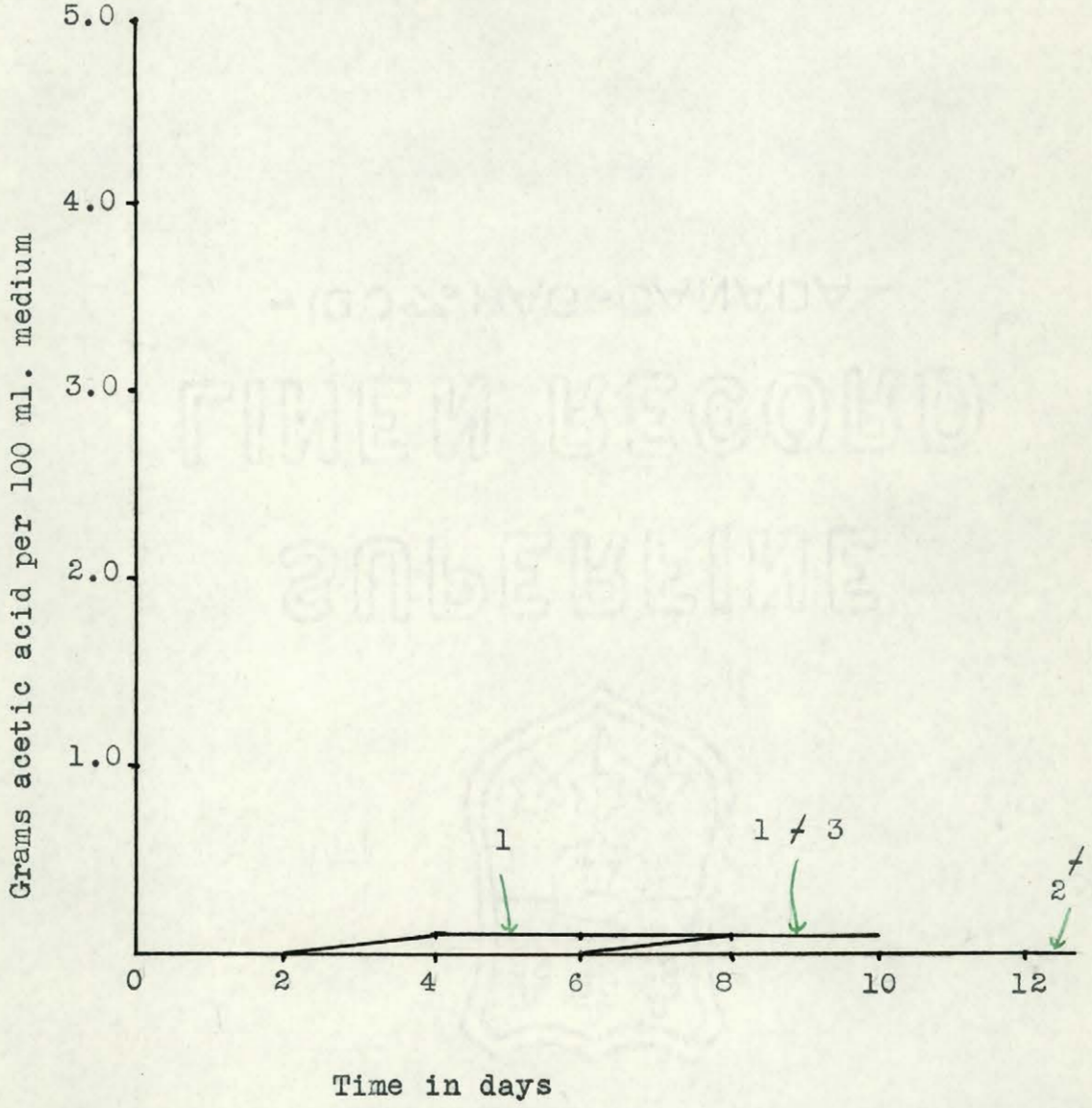


FIGURE 51 - THE EFFECT OF MIXED INOCULA IN THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER TURBIDANS

Curve 1 - Mixture, A. turbidans ≠ A. aceti 1
2 - A. turbidans
3 - A. aceti 1

≠ - All readings 0.

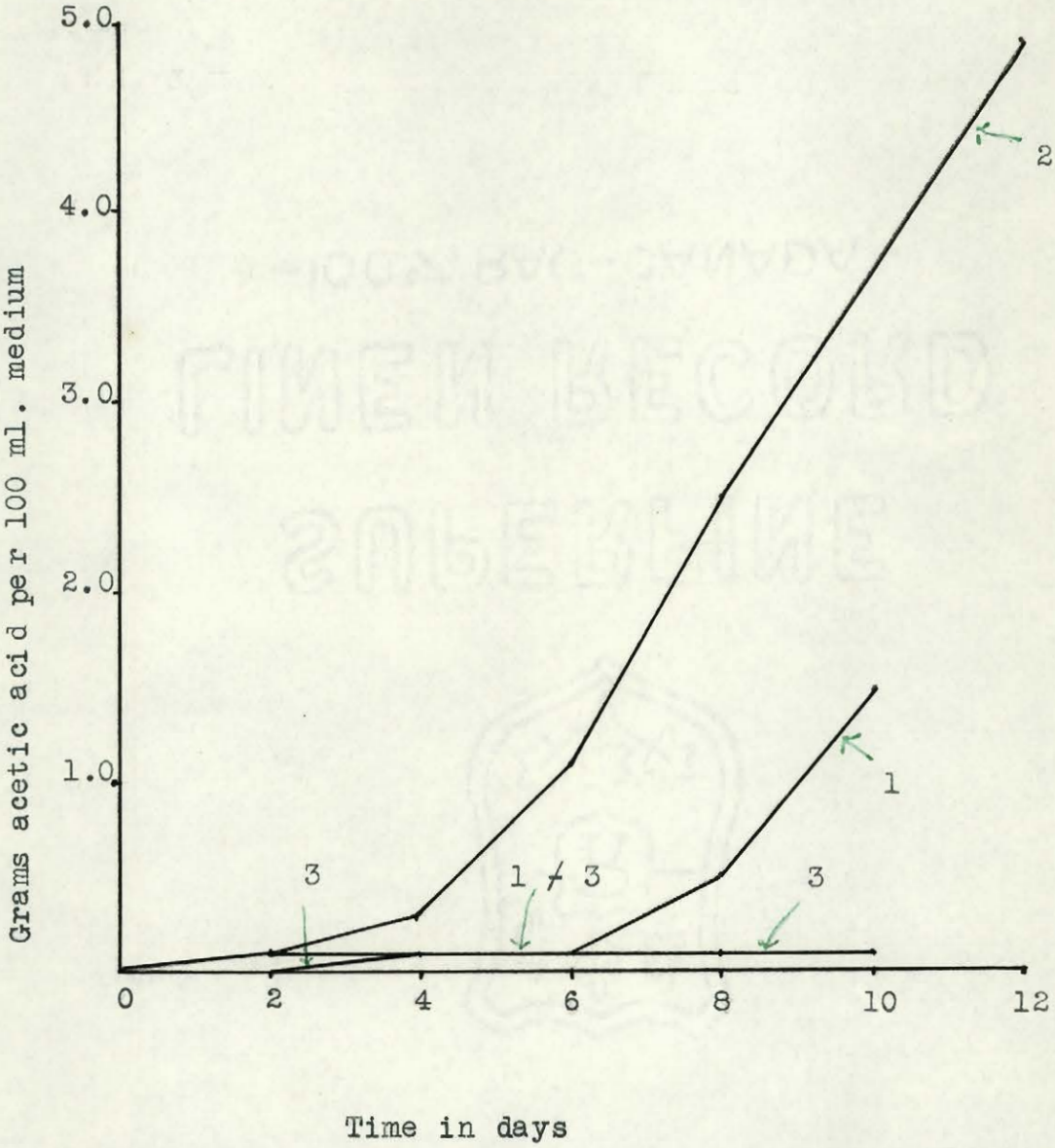


FIGURE 52 - THE EFFECT OF MIXED INOCULA IN THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED - ACETOBACTER KÜTZINGIANUM

Curve 1 - Mixture, A. kützingianum + A. aceti 1
2 - A. kützingianum
3 - A. aceti 1

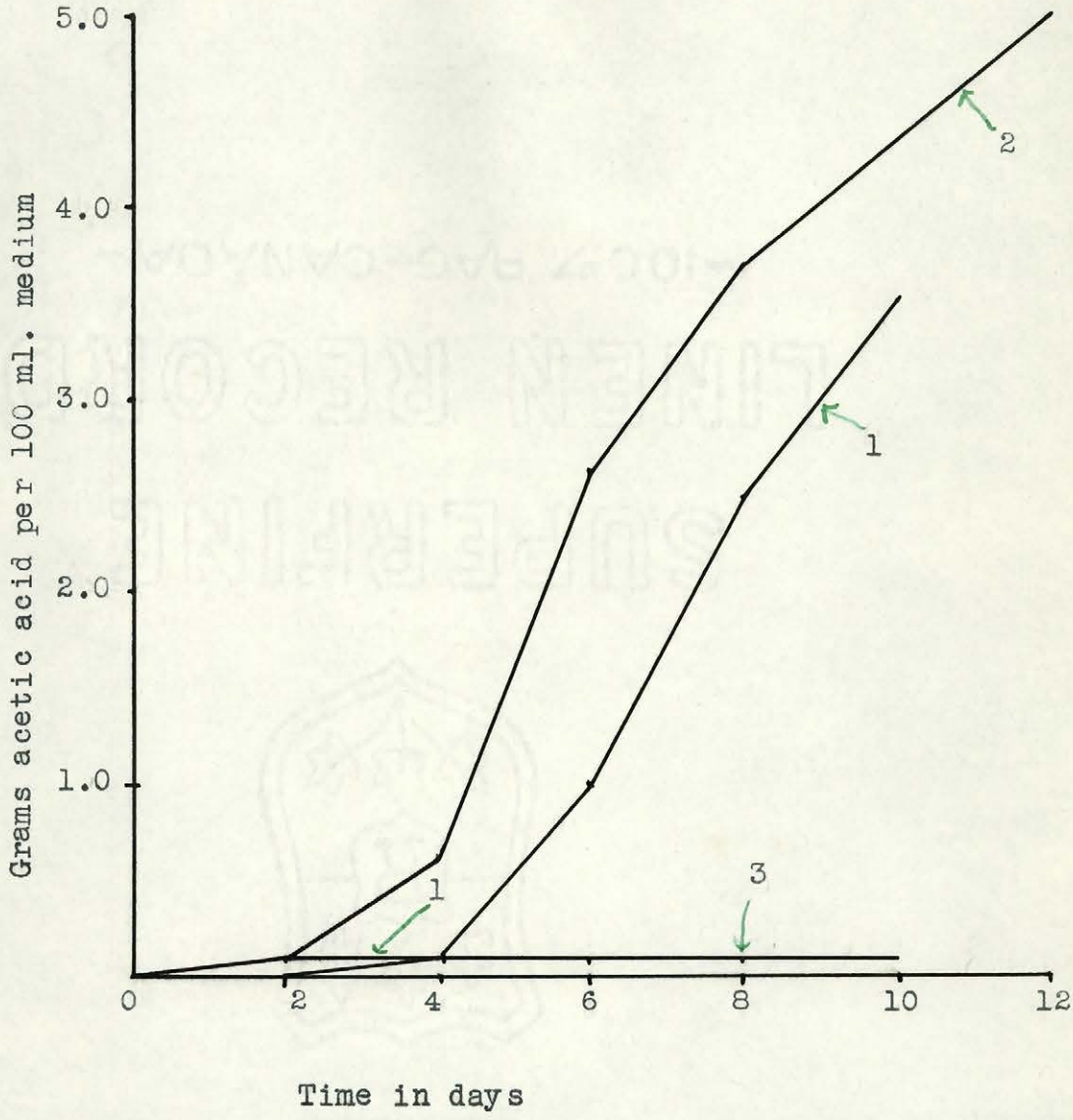


FIGURE 53 - THE EFFECT OF MIXED INOCULA IN THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER ACETI 2

Curve 1 - Mixture, A. aceti 2 + A. aceti 1
2 - A. aceti 2
3 - A. aceti 1

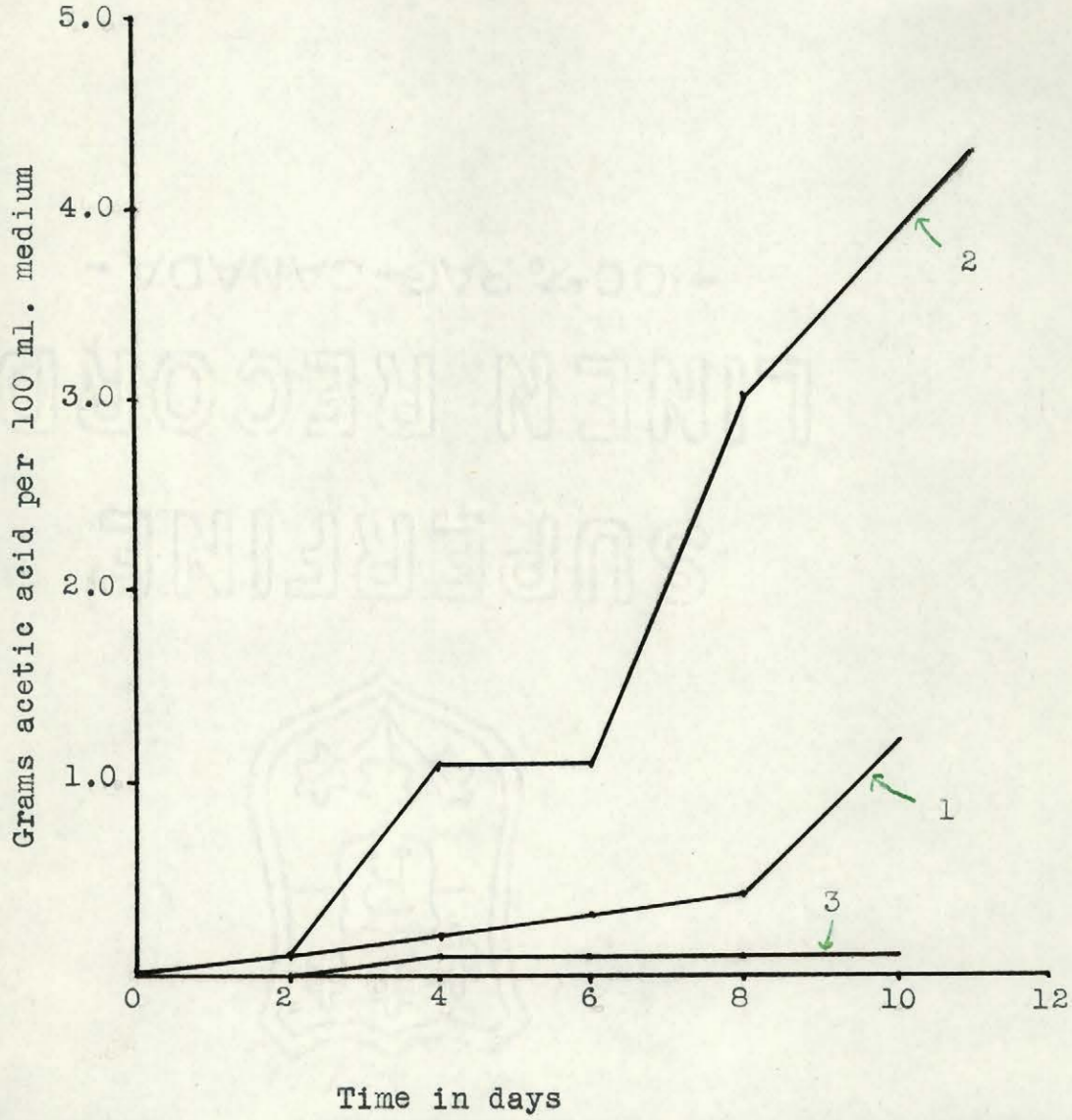


FIGURE 54 - THE EFFECT OF MIXED INOCULA IN THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER RANCENS

Curve 1 - Mixture, A. rancens + A. aceti 1
2 - A. rancens
3 - A. aceti 1

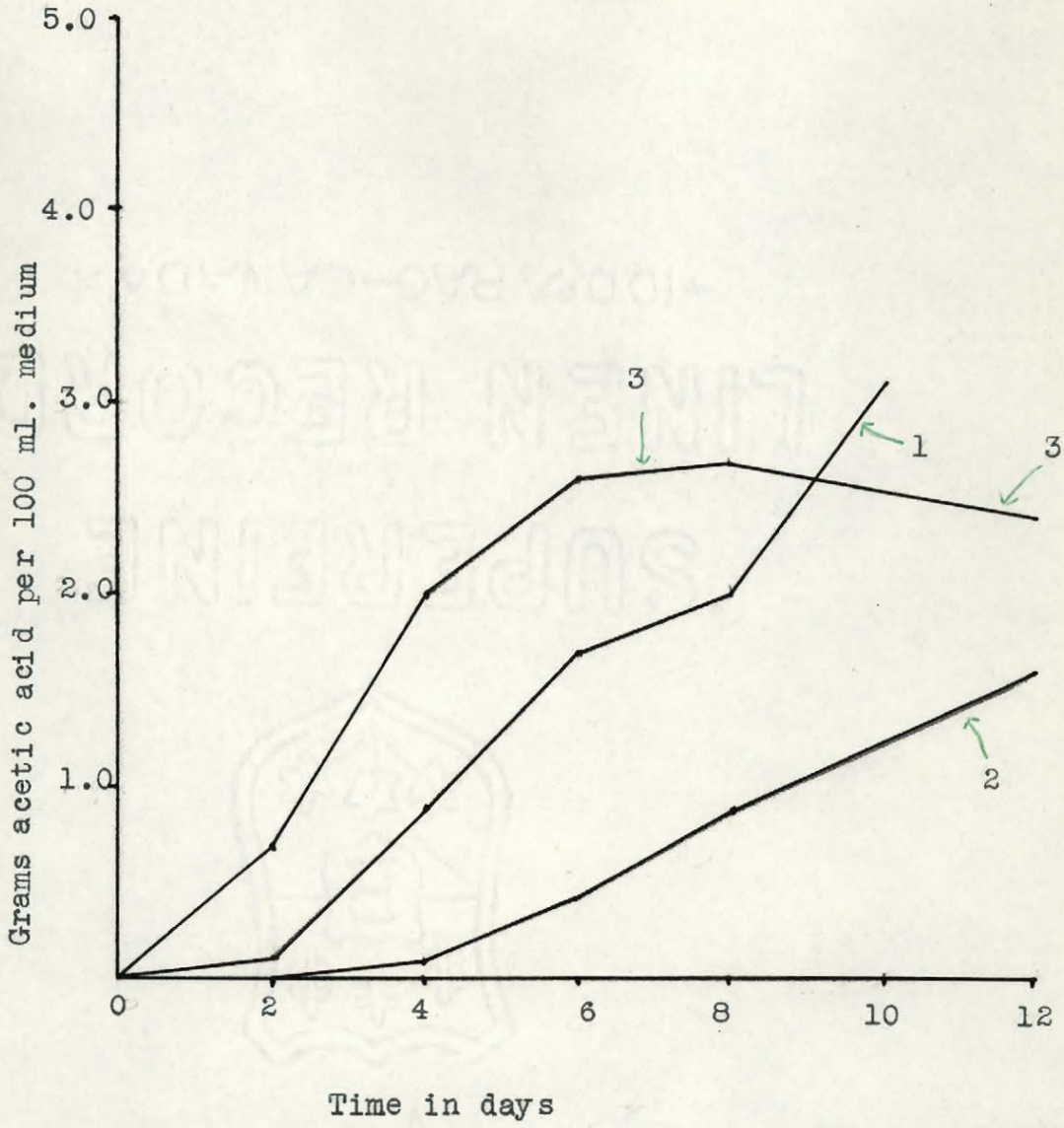


FIGURE 55 - THE EFFECT OF MIXED INOCULA IN THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED - ACETOBACTER GLUCONICUM

Curve 1 - Mixture, A. gluconicum + A. aceti 1
2 - A. gluconicum
3 - A. aceti 1

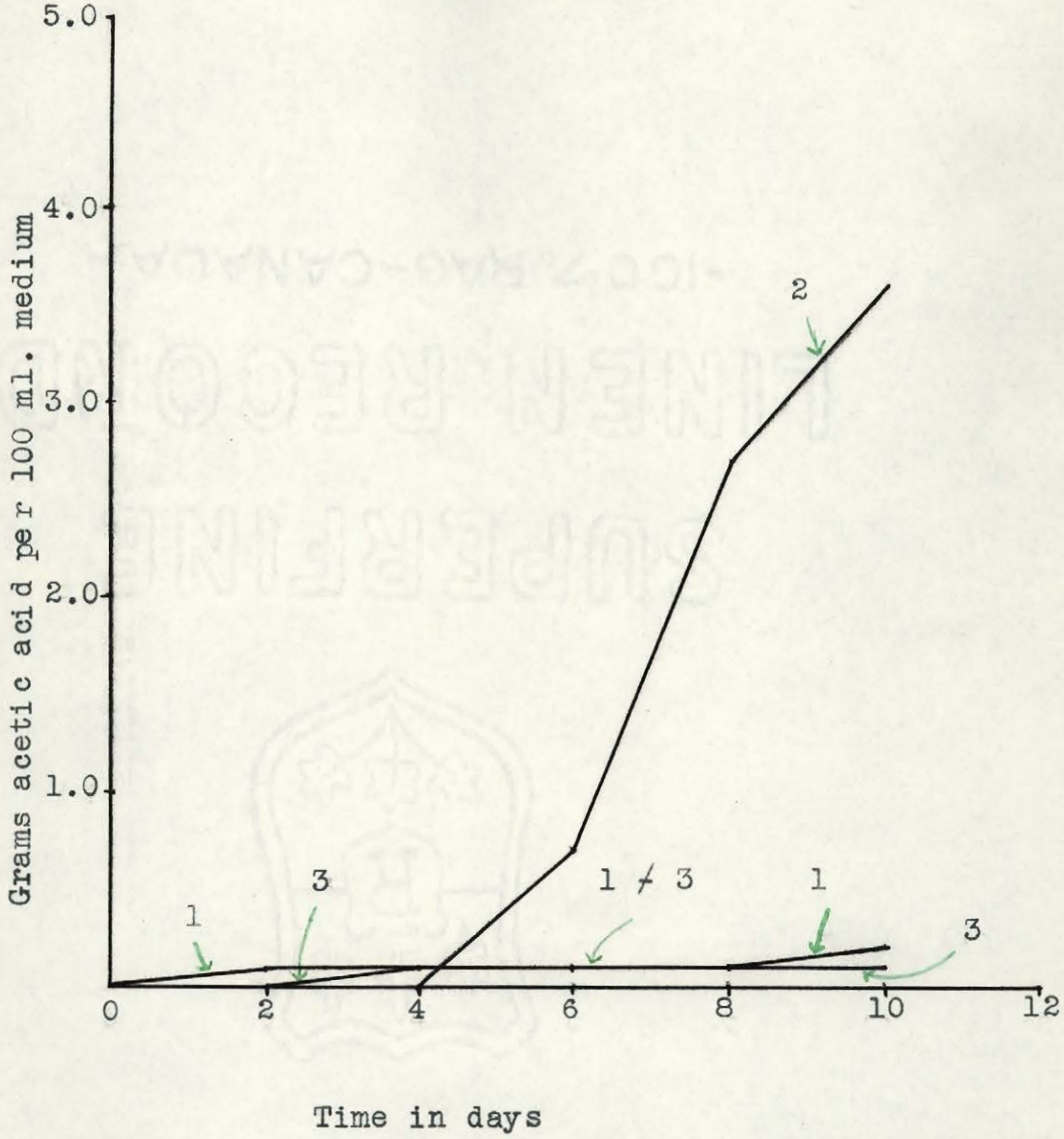


FIGURE 56 - THE EFFECT OF MIXED INOCULA IN THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED - ACETOBACTER MELANOGENUM 1

Curve 1 - Mixture, A. melanogenum 1 + A. aceti 1
2 - A. melanogenum 1
3 - A. aceti 1

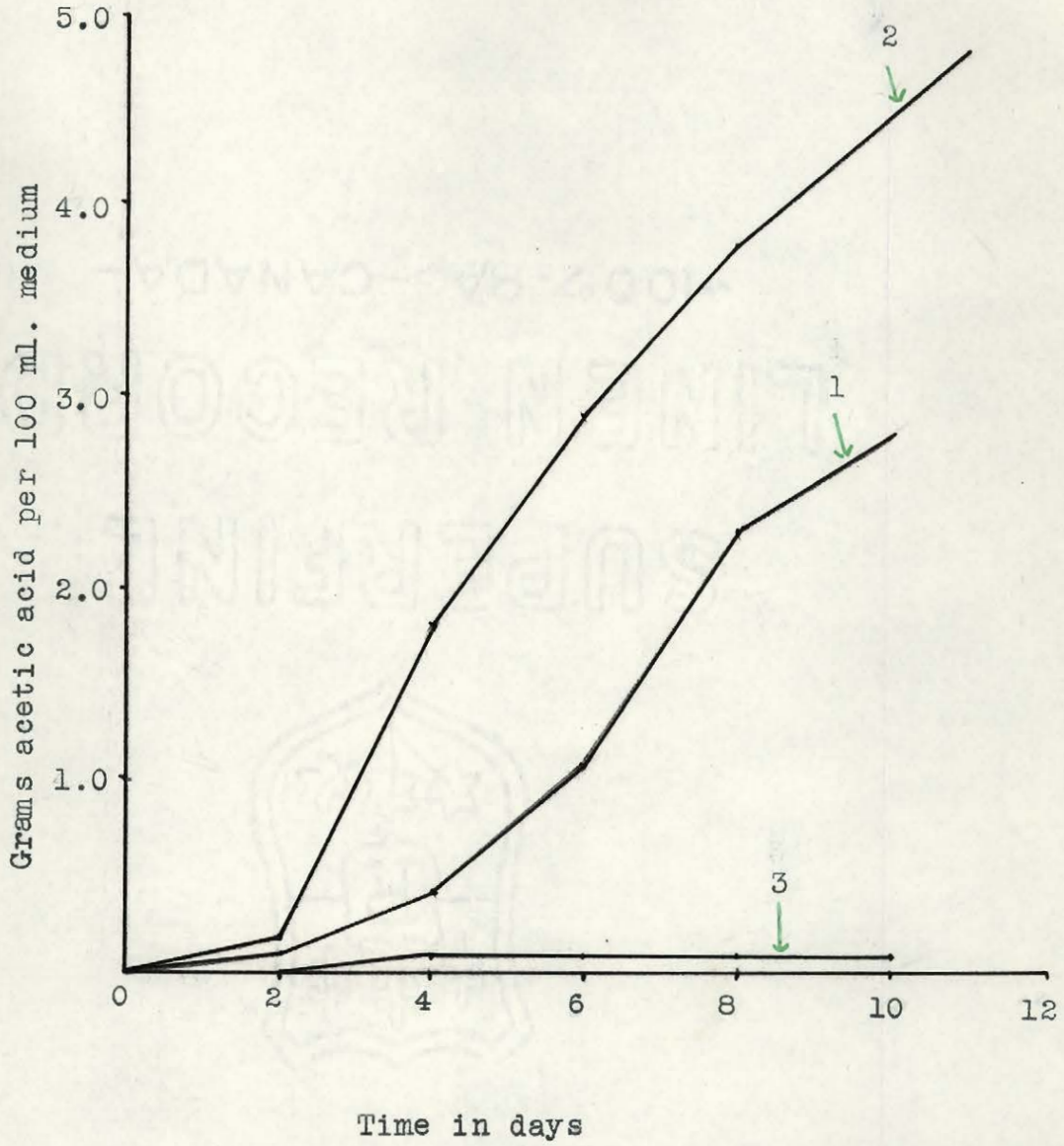


FIGURE 57 - THE EFFECT OF MIXED INOCULA IN THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER OXYDANS 1

Curve 1 - Mixture, A. oxydans 1 + A. aceti 1
2 - A. oxydans 1
3 - A. aceti 1

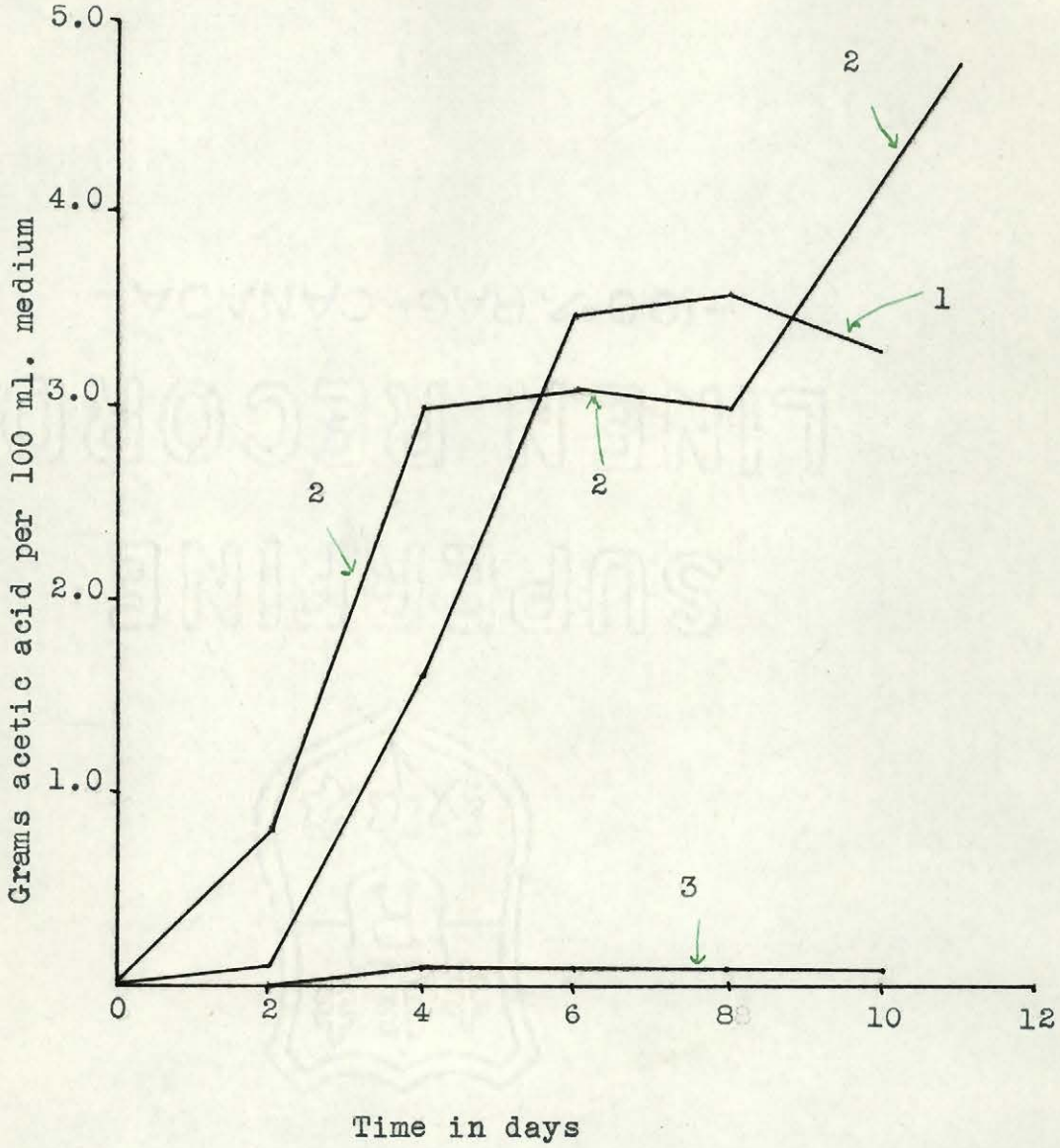


FIGURE 58 - THE EFFECT OF MIXED INOCULA IN THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER OXYDANS 2

Curve 1 - Mixture, A. oxydans 2 + A. aceti 1
2 - A. oxydans 2
3 - A. aceti 1

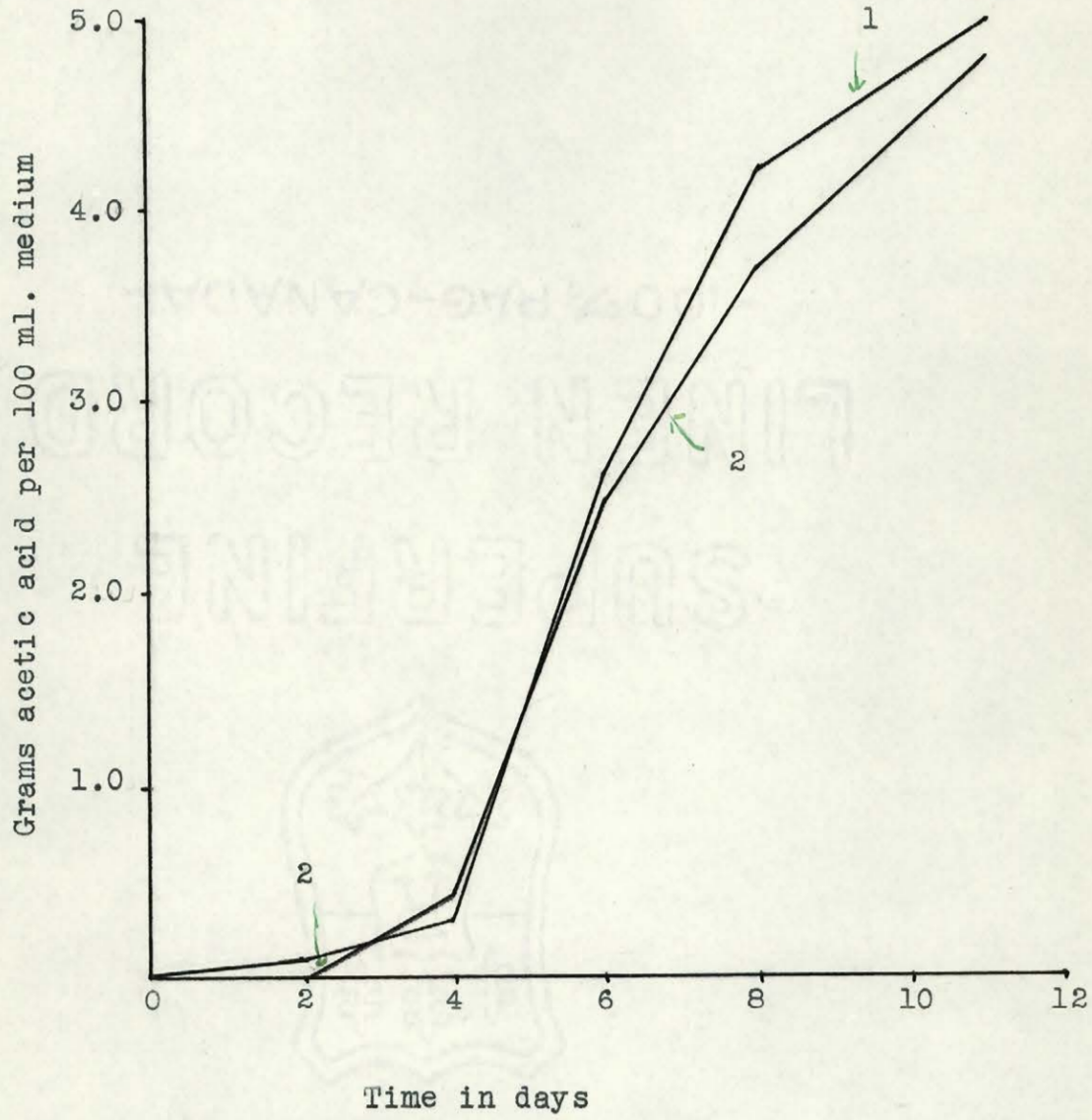


FIGURE 59 - THE EFFECT OF MIXED INOCULA IN THE YEAST EXTRACT MEDIUM ON THE RATE OF ACETIC ACID PRODUCED - ALL ACETOBACTER SPECIES TOGETHER

Curve 1 - Mixture, all Acetobacter organisms
2 - A. oxydans 1 from Appendix Table XXI
(Rate of acid formation by individual organism similar to that of all organisms together)

Media containing 6 per cent ethyl alcohol were inoculated with a mixture of Acetobacter kützingianum, Acetobacter aceti 2, Acetobacter rancens, Acetobacter melanogenum 1, Acetobacter oxydans 1 or Acetobacter oxydans 2 and Acetobacter aceti 1. Acetobacter melanogenum 1, which was very spasmodic in acid production, produced a significant quantity of acetic acid only when the organism alone was used as inoculum. Other mixed inocula produced a lag in acid production when compared with the rate of production by the individual organisms, although the same types of curves were produced. Mixed inocula produced an acceleration in acid production when compared with the rate of production by Acetobacter aceti 1. Each organism alone, that is other than Acetobacter aceti 1, produced a maximum amount of acetic acid.

Media containing 3 per cent ethyl alcohol were inoculated with a mixture of Acetobacter suboxydans, Acetobacter acetosum, Acetobacter ascendens, Acetobacter pasteurianum or Acetobacter gluconicum and Acetobacter aceti 1. Acetobacter suboxydans and Acetobacter pasteurianum were not discussed previously in terms of acetic acid production because they produced insignificant quantities of acid. A mixed inoculum of Acetobacter suboxydans and Acetobacter aceti 1 produced 0.2 gm. of acetic acid; a mixed inoculum of Acetobacter pasteurianum and Acetobacter aceti 1 produced acetic acid at a slower rate than Acetobacter aceti 1, but the same amount. A mixed inoculum of Acetobacter gluconicum, which was very spasmodic in acid production, and Acetobacter aceti 1 produced acid at a faster rate than Acetobacter gluconicum alone; however the rate was

not as fast as that of Acetobacter aceti 1 alone. The mixed inoculum produced significantly more acid than either organism alone. A mixed inoculum of Acetobacter acetosum or Acetobacter ascendens and Acetobacter aceti 1 produced acid at a rate somewhat similar to that of each component of the inoculum and either mixture produced a maximum average amount of acetic acid that was insignificantly different from that produced by the components.

When comparisons were made between the rate of acid production by Acetobacter aceti 1 in combination with the organisms and alone, the relevant Acetobacter aceti 1 results were taken from the closest possible test under the conditions of the present test. Thus, where 3 per cent ethyl alcohol and an incubation temperature of 25° or 30° C. were used the results from the effect of the appropriate pH were used; if, however, an incubation temperature of 35° C. was used the results were taken from the effect of that temperature, since acetic acid production was much less. Where 6 or 9 per cent ethyl alcohol was used the corresponding results were taken, since insignificant amounts of acid were formed and the yield would not be influenced by the pH of the media or the incubation temperature.

A mixture of all organisms:

When the inoculum consisted of all organisms the maximum average amount of acid produced was comparable with that produced by the individual organisms producing the maximum amounts of acetic acid. The rate of production was compared with that produced by Acetobacter kützingianum, Acetobacter aceti 2,

Acetobacter oxydans 1 and Acetobacter oxydans 2 and was found to be most like the rate produced by Acetobacter oxydans 1 in 6 per cent ethyl alcohol medium adjusted to pH 7.0 and incubated at 25° C. Acetobacter aceti 2 also produced acid at a somewhat similar rate in 6 per cent ethyl alcohol medium adjusted to pH 5.5 and incubated at 25° C.

4. The Effect of Varying Chemical Factors on the Amount of Acetic Acid Produced

The results of the experiments are recorded in Figures 60 to 63 (pp.219-222), and Appendix Tables XXXI to XXXIV (pp.275-278) and Appendix Table XXIII (p.267).

Acetobacter acetosum, Acetobacter ascendens, Acetobacter aceti 2, Acetobacter rancens, Acetobacter gluconicum and Acetobacter melanogenum 1 produced insignificant amounts of acetic acid in all synthetic media.

Acetobacter kützingianum produced 0.3 gm. acetic acid per 100 ml. in a medium consisting of inorganic salts, amino acids and vitamins. When pyrimidine and purine bases were added 0.23 gm. was produced.

The other organisms produced considerable lag in acid production when oxidizing ethyl alcohol in synthetic media. The least amount of lag was observed with Acetobacter aceti 1 and Acetobacter oxydans 2 in a medium containing inorganic salts and amino acids. Synthetic media changed the characteristic curve of the rate at which Acetobacter oxydans 2 produced acetic acid.

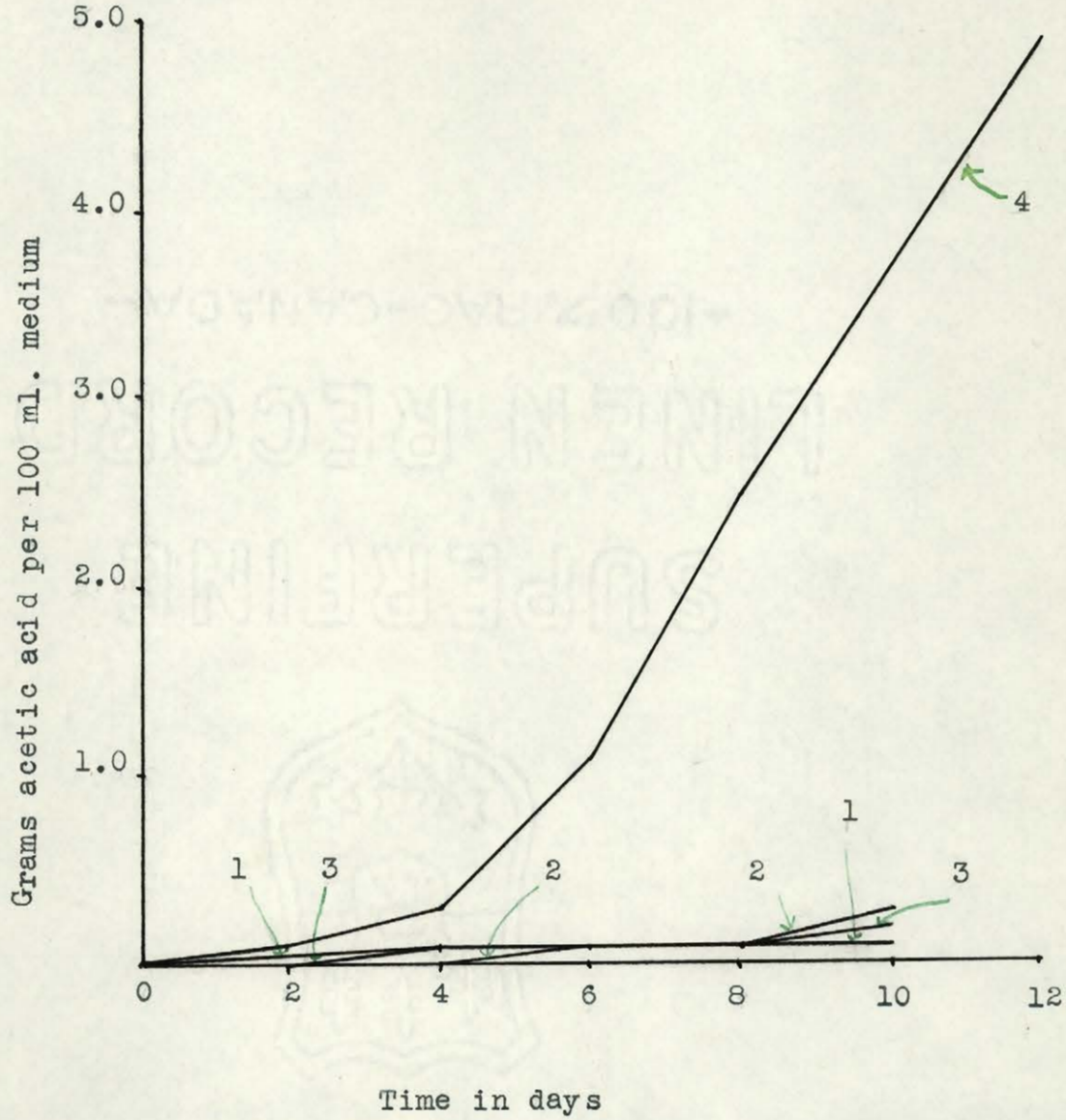


FIGURE 60 - THE EFFECT OF VARYING THE CHEMICALS IN THE MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER KUTZINGIANUM

Curve 1 - Medium I, inorganic salts + amino acids
2 - Medium II, inorganic salts + amino acids + vitamins
3 - Medium III, inorganic salts + amino acids + vitamins + pyrimidine and purine bases
4 - Medium IV, yeast extract

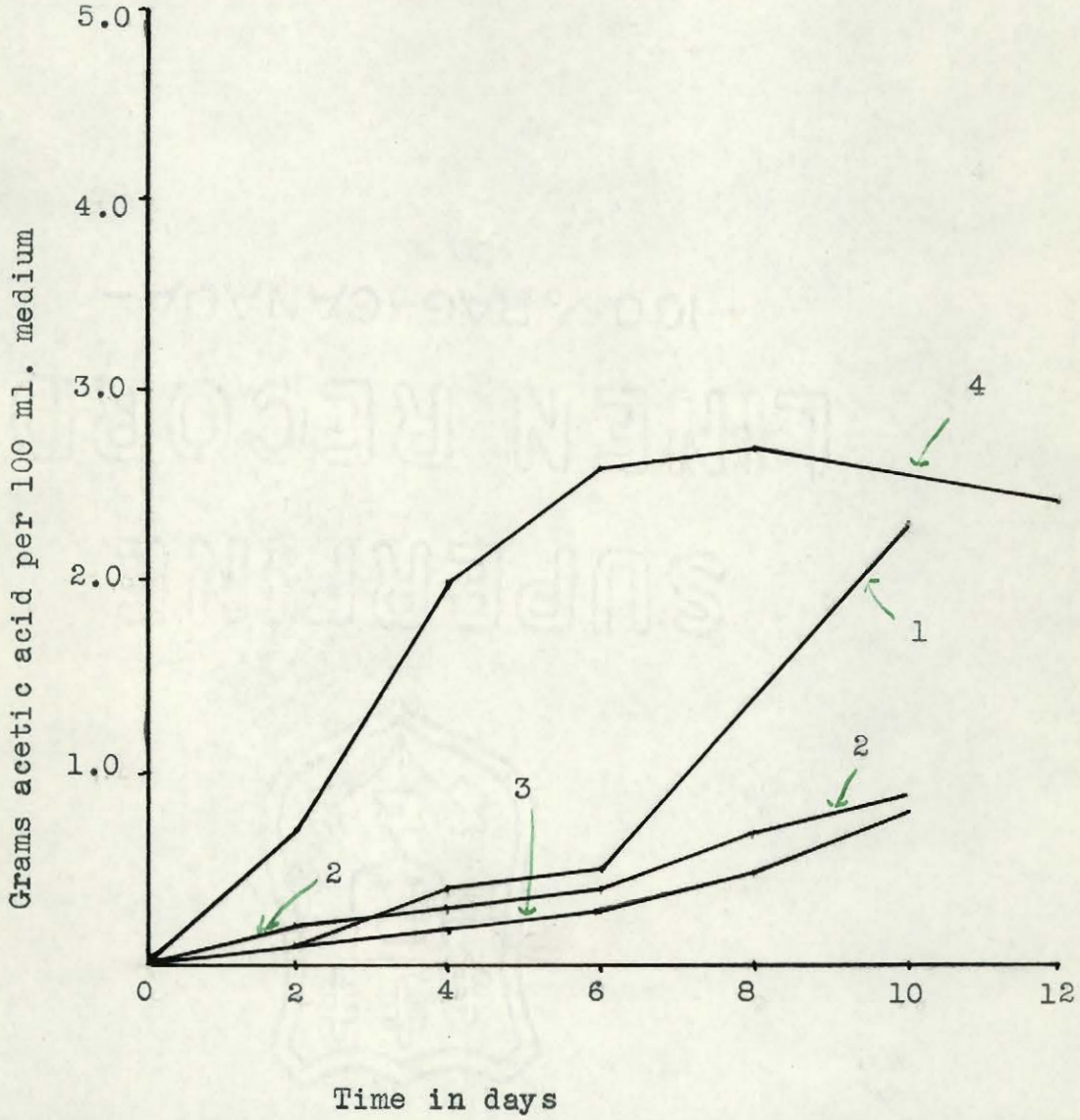


FIGURE 61 - THE EFFECT OF VARYING THE CHEMICALS IN THE MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER ACETI 1

Curve 1 - Medium I, inorganic salts + amino acids
2 - Medium II, inorganic salts + amino acids + vitamins
3 - Medium III, inorganic salts + amino acids + vitamins + pyrimidine and purine bases
4 - Medium IV, yeast extract

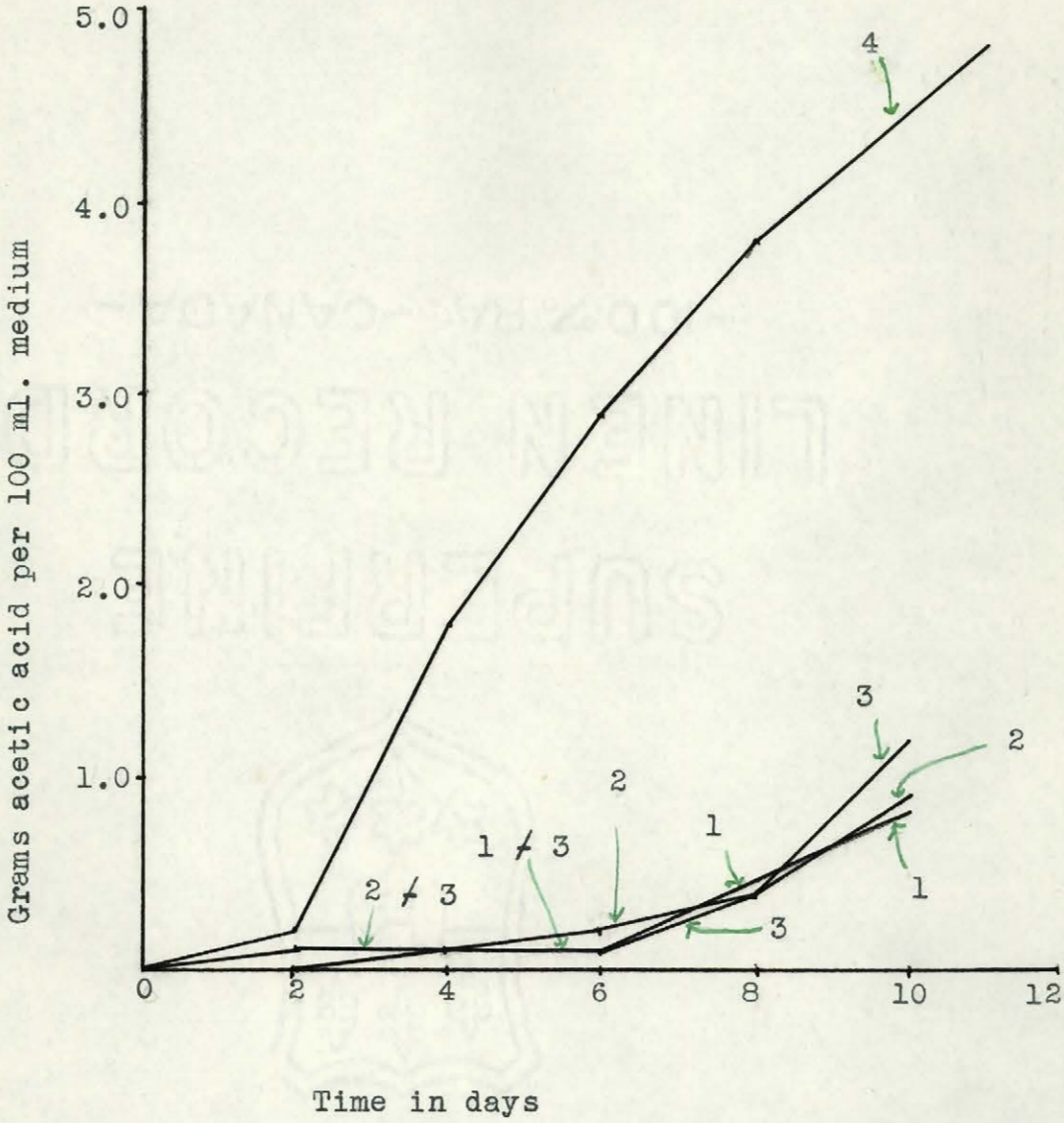


FIGURE 62 - THE EFFECT OF VARYING THE CHEMICALS IN THE MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER OXYDANS 1

Curve 1 - Medium I, inorganic salts ∕ amino acids
2 - Medium II, inorganic salts ∕ amino acids ∕ vitamins
3 - Medium III, inorganic salts ∕ amino acids ∕ vitamins ∕ pyrimidine and purine bases
4 - Medium IV, yeast extract

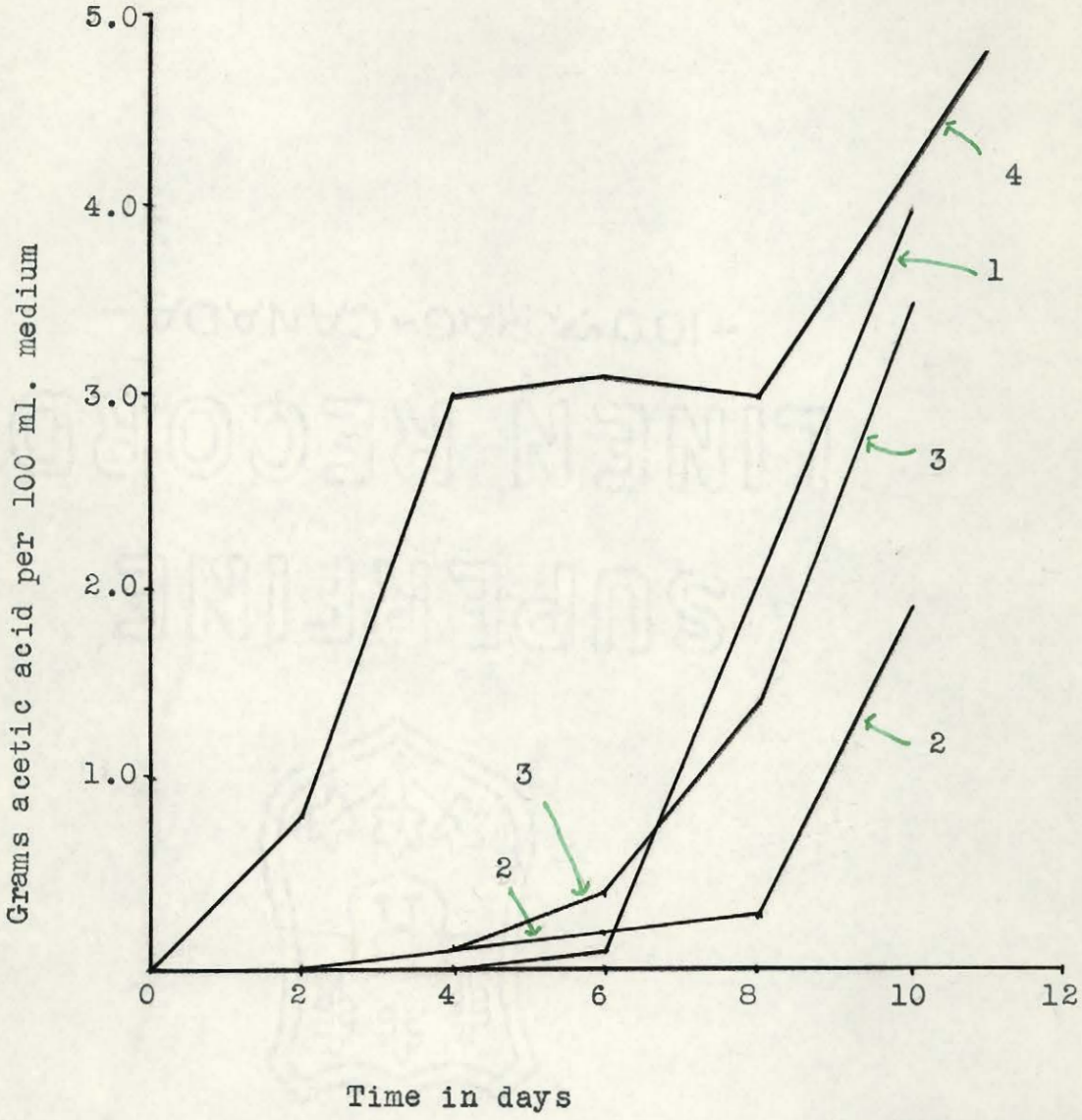


FIGURE 63 - THE EFFECT OF VARYING THE CHEMICALS IN THE MEDIUM ON THE RATE OF ACETIC ACID PRODUCED BY ACETOBACTER OXYDANS 2

Curve 1 - Medium I, inorganic salts + amino acids
2 - Medium II, inorganic salts + amino acids + vitamins
3 - Medium III, inorganic salts + amino acids + vitamins + pyrimidine and purine bases
4 - Medium IV, yeast extract

In terms of variations producing a maximum amount of acetic acid, Acetobacter aceti 1 produced insignificantly different amounts of acetic acid in a synthetic medium consisting of inorganic salts and amino acids and in yeast extract medium. Acetobacter oxydans 2 produced a good maximum average yield of acid in a synthetic medium consisting of inorganic salts and amino acids, but produced the greatest yield in yeast extract medium. Acetobacter oxydans 1 produced smaller maximum yields of acid in synthetic media. Insignificantly different maximum amounts of acid were produced in a medium containing inorganic salts, amino acids and vitamins and in a medium containing inorganic salts, amino acids, vitamins and pyrimidine and purine bases.

During one part of the experiment to test the effect of Medium III on acid production by Acetobacter species, test flasks became cloudy from bacterial growth even when acetic acid was not produced. The group of organisms observed included Acetobacter turbidans, Acetobacter kützingianum, Acetobacter aceti 2, Acetobacter rancens, Acetobacter melanogenum 1, Acetobacter oxydans 1 and Acetobacter oxydans 2. Only the medium containing Acetobacter turbidans did not become cloudy. Three of the remaining six organisms produced insignificant amounts of acid. It is apparent, therefore, that the nutritional conditions for growth are different than those required for acid production. Possibly an unidentified substance or substances in yeast extract must be supplied to the organisms for synthesis of the enzymes that bring about oxidation of alcohol, or a

substance or substances in the synthetic media must be omitted for the synthesis of the enzymes because of their inhibitory effect.

G. DISCUSSION AND CONCLUSIONS

The decision to allow the control flasks to contain the same fermenting media as the test flasks without ethyl alcohol was apparently the only feasible one. Products from metabolic processes other than acetic acid production must be formed by the organisms. However, products may not necessarily be by-products of the fermentation, but indirectly associated with the general metabolism when the Acetobacter organisms are oxidizing ethyl alcohol. That is, the relationship of reactions in the presence of alcohol may be different from that in its absence. Dried brewer's yeast is largely protein, 40 to 55 per cent; and most of the nitrogen free content is a mannose polysaccharide (54). Difco's Bacto-Yeast Extract is the water soluble portion of autolyzed yeast. During autolysis the proteins produce peptides and amino acids; and the polysaccharide may produce mannose. In alcohol-omitted media, the organisms must rely on a mannose-type sugar and amino acids as a source of carbon. The products formed in metabolic processes may be quite different, therefore, from those formed when ethyl alcohol is present. Even a different attack by the organisms on amino acids may affect the pH of the medium differently. The sample titrations of inoculated test and control media indicate how the organisms change the media in terms of acid production, when alcohol is and is not present. Differences in metabolic activity do not necessarily affect media similarly in terms of acid

production. Interestingly, the amount of acid present in the control fermenting media during fermentation was sometimes slightly greater than that in the test fermenting media when vitamins were used in the chemical basic synthetic medium. During all tests the titrations of samples from the control fermenting media were negligible. All acid yields represented the free acid content. Although an attempt was made to separate the acetic acid from the total acid content in test fermenting media, the method cannot be considered very reliable. Methods of identifying compounds directly in fermenting test media would be better.

As stated on page 110 eleven or twelve-day samples sometimes were taken in place of ten-day samples. The eleven or twelve-day sample titrations were allowed to replace those of the ten-day due to the possible exhaustion of the nutrients and the evaporation of the ethyl alcohol and acetic acid in the media in the extra time. If an organism produced a maximum average yield of acetic acid in the twelve-day interval with one variation of a factor and that yield was greater than the maximum yield produced by the same organism with another variation of the factor in the ten-day interval, the twelve-day reading determined the variation of the factor that would be used in the next experiment, no matter what the ten-day reading may have been. In making the figures of the rate of acid production an eleven or twelve-day reading was plotted where necessary to determine what the acid content could have been if the concentration of the nutrients, ethyl alcohol and

acetic acid were sufficient. In several described instances the maximum average yield in the ten-day interval was lower than the maximum average yield produced by other variations of the same factor. Acetobacter kützingianum produced 3.70 gm. acid per 100 ml. of medium in the ten-day interval and 4.87 gm. in the twelve-day interval at pH 5.5. Other maximum acid yields produced by the organism with variations of pH were illogical. When comparing the results obtained with increasing pH there was no increasing or decreasing trend in acid production. The great difference in acid production was possibly due to the spasmodic production of acid by the organism. Slight differences appeared in the maximum average acid yields with other organisms, but the differences were insufficient to change the pH that would be used for the organisms in succeeding experiments. The only other instance of changing maximum averages for factors studied was produced by the mixed inoculum of Acetobacter ascendens with Acetobacter aceti 1; the difference was too small to even warrant mention. Variations of pH in the highest acid-producing category changed for Acetobacter kützingianum, Acetobacter aceti 2, Acetobacter rancens, Acetobacter oxydans 1 and Acetobacter oxydans 2 when ten-day instead of eleven or twelve-day samples were taken. These organisms were classified as being spasmodic in acid production, except Acetobacter aceti 2. Whether ten, eleven or twelve-day samples were taken the groupings of pH's that produced the same effect on acid production by the organisms were illogical. Therefore, possibly the differences in maximum average yields

of acetic acid with different pH's were influenced more by spasmodic behavior than by change in pH. Variations in the highest acid-producing category of maximum average yields changed slightly with Acetobacter ascendens in studying the effect of the size of the inocula, and more with Acetobacter oxydans 2 in studying the effect of chemical factors. Taking all differences into consideration, the eleven or twelve-day sample could replace the ten-day sample.

Acetobacter species decidedly varied in their ability to produce acetic acid continuously. Even those organisms that did not produce a significant amount of acid may be able to produce acid under different circumstances. All Acetobacter organisms were propagated on a medium in which glucose was the major source of carbon; inocula were prepared from cultures grown on the same medium. The organisms may have lost their ability, in varying degrees, to utilize ethyl alcohol. All the organisms at some time must have produced acetic acid in order to have been placed within the genus. By training on media containing ethyl alcohol possibly all organisms would be later capable of producing acid. Their final rate of acid production may be superior to that of less spasmodic acid producers. Two examples of adaptation of the organism to utilize ethyl alcohol may be cited. Acetobacter suboxydans when first procured produced good quantities of acetic acid during fermentation, and later did not produce acid. All organisms that produced significant quantities of acid

produced a varying degree of lag in acid production when the fermenting media initially contained 6 per cent ethyl alcohol instead of 3 per cent. Using media containing 9 per cent ethyl alcohol, all organisms produced less than 0.2 gm. acetic acid except Acetobacter oxydans 1 and Acetobacter oxydans 2, which produced a maximum yield of 0.21 and 2.3 gm. of acetic acid per 100 ml., respectively, in ten days. Acetobacter oxydans 2 gave a much greater lag in acid production when the initial ethyl alcohol concentration was 9 per cent instead of 6 per cent. Each of five organisms produced the greatest maximum yield of acid in 6 per cent alcohol-containing media. With the above-mentioned results the possibility exists of training Acetobacter species to produce good yields of acid in 9 per cent or even 12 per cent alcohol-containing media. As suggested in the literature, however, Acetobacter species vary in their alcohol tolerance, and the higher the alcoholic concentration the greater the degree of lag in acid production.

Some organisms, especially with certain variations of factors, produced a maximum average amount of acid in a short interval of time, and at a later time the average amount became smaller. At first the drop in acid content was thought to have been due to the oxidation of acetic acid to carbon dioxide and water by the organisms. However not all of these organisms oxidize acetic acid. Therefore, in some instances the drop in the amount of acid must have been due to the conversion of acid into other substances; the inability

of the organisms to function satisfactorily in the amount of acid produced; or the inability of the replacement broth to supply sufficient nutrients in order that the bacteria could maintain the amount of acid. The organisms, however, must be able to function satisfactorily in the amount of acid produced because they produced greater quantities of acid in 6 per cent than in 3 per cent ethyl alcohol-containing media, although the average amount of acid formed in 3 per cent alcohol-containing media became smaller. Possibly the amount of alcohol added at the required intervals was insufficient to maintain the amount of acid produced. Also, since in other instances no drop in the quantity of acid produced occurred at later sampling-times, possibly sufficient nutrients, other than ethyl alcohol, were present. It is possible that the rates of metabolism were hindered in the beginning and therefore the supply of nutrients was greater later. Also, as suggested in the literature, species vary in their ability to oxidize acetic acid and all alcohol must be converted to acetic acid before the acetic acid is converted to carbon dioxide and water.

Variations of pH could not be grouped together logically as most favourable for a maximum average production of acetic acid especially with the spasmodic acid-producing organisms. When comparing the results obtained with increasing pH, an increasing or decreasing trend in acid production was not evident. Even the lag in acid production with some organisms when the initial pH of the media was 6.0 was insignificant.

Changes in the organisms could have occurred because the experiment was done before the others. The only conclusion is that illogical changes in maximum acid production with media at different initial pH's are due to the fact that the influence of spasmodic acid production is greater than the influence of pH.

As was stated previously, yeast extract from Difco was used during all experiments with the exception of those in which the effect of the size of the inocula and mixed cultures on acid production was being studied. As the results of the effect of inocula prepared from one slope were taken from the previous experiment, the fermenting media contained Difco's yeast extract. The fermenting media for all other experiments were made with yeast extract from the Nutritional Biochemicals Corporation. With the experiments on the effect of mixed inocula the results on acid production by each individual organism were taken from the same experiment, and other experiments in which Difco's yeast extract was used. Organisms were also cultured on yeast extract from the Nutritional Biochemicals Corporation. Organisms did not grow well on the culture medium containing the latter substance. As time passed their growth became more scant, indicating that they could not adapt themselves to the new medium. The results of the final group of experiments indicated that Acetobacter vary in their nutritional requirements. Evidently the two types of yeast extract do not contain the same substances and would not affect all organisms in the same way.

When the latter fact became evident too much time had elapsed in order that the experiments could have been significant in relation to all of the others if repeated with Difco's yeast extract. The better approach would be to compare the effect of inocula prepared from three and five slopes on acid production. Inocula from three and five-slope cultures of each organism produced insignificantly different amounts of acetic acid, and the rates of acid production were very similar. Taking the change of medium into consideration, the effect of each organism alone in acetic acid production did not influence the effect of the combination of organisms in acid production. For example, Acetobacter suboxydans produced little acid either alone or in combination with Acetobacter aceti 1, but the latter organism produced acid even in the presence of the inferior type of yeast extract, and was a continuous acetic acid producer.

From the results of the experiments on the effect of chemical factors on acetic acid production, Acetobacter differ in their nutrient requirements. Some organisms were capable of growing in the various media but did not produce acid, as in yeast extract media. Possibly, therefore, even yeast extract may not contain the proper balance of nutrients to allow certain Acetobacter species to produce acid. Acetobacter organisms did not produce acid in synthetic media at the rate produced in yeast extract media. Acetobacter aceti 1 produced insignificantly different maximum amounts of acetic acid in a

synthetic medium consisting of inorganic salts and amino acids and in the corresponding yeast extract medium. The maximum amount of acid produced in a medium containing vitamins or vitamins and pyrimidine and purine bases was significantly less, and production was at a slower rate. Therefore the substances interfered with the metabolic processes required during fermentation. Acetobacter oxydans 2 oxidized ethyl alcohol in a medium consisting of inorganic salts and amino acids well. Vitamins caused less acid to be formed, and vitamins in the presence of pyrimidine and purine bases were less inhibitory. Acetic acid fermentation, therefore, is related to the individual nutrient requirements of the organism. The components of the synthetic media used during experiments were not meant to replace those of yeast extract. The components were used because they were previously found to affect growth of Acetobacter organisms. Wilder and Keys (54) have reviewed the nutrients present in dried brewer's yeast. It is largely protein, 40 to 55 per cent. Among the amino acids found in the proteins are alanine, valine, phenylalanine, glutamic acid, aminoacetic acid, leucine, oxyproline, aspartic acid, cystine, methionine, tyrosine, proline and tryptophan. The percentage of these ranges from 10 to 0.5 per cent in the order given. The diamino acids present include lysine 10 per cent, arginine 5 per cent and histidine 5 per cent. The content of vitamins of the B complex is very great. Some vitamin A is present and much

ergosterol. The amount of fat is small, from 1 to 3.5 per cent, but this is rich in steroids. Most of the nitrogen free content is a mannose polysaccharide. Peterson (25) maintained that yeast is one of the richest sources of the B vitamins. The vitamin content varies greatly because of differences in the medium in which the yeast is grown. Brewer's yeast contains thiamine, riboflavin, nicotinic acid, pantothenic acid, pyridoxine, p-aminobenzoic acid, folic acid and biotin. Baldwin (3) claimed that nucleic acids in yeasts contain cytosine, uracil, adenine and guanine. Peterson (25) maintained that yeast is a superior source of phosphorus and iron, and compares favourably with many staple foods in calcium content. The method by which yeast is autolyzed determines the final chemical composition.

H. SUMMARY

A. Individual organisms vary in the rate at which they produce similar amounts of acetic acid under identical conditions. Spasmodic production of acetic acid is thought to be a species characteristic. Some Acetobacter species did not produce acetic acid. Only organisms that produced acid continuously are discussed.

B. Acetic acid production in yeast extract medium:

1. All Acetobacter produced a lag in acid production when fermenting broth was incubated at 12° to 24° C., and some produced a lag at 35° C. All organisms oxidized ethyl alcohol satisfactorily at 25° C.
2. All Acetobacter tended to produce a greater lag in acid production when the surface area of the fermenting liquid was 43.9 instead of 76.6 sq.cm. In terms of maximum acid production by each organism, all organisms oxidized alcohol satisfactorily when the surface area at the meniscus of the liquid was the smaller.
3. In general, organisms did not produce a lag in acid production when two, four, six, eight and ten-day samples were taken instead of three, five and eight-day samples. Each organism produced insignificantly different maximum amounts of acid with the two types of sampling-time intervals.

4. All Acetobacter produced a varying degree of lag in acid production when the medium initially contained 6 per cent instead of 3 per cent ethyl alcohol, and an even greater lag when the medium contained 9 per cent. Some organisms produced insignificant amounts of acid with 6 per cent or 9 per cent ethyl alcohol or both. Some organisms produced a maximum amount of acid in 3 per cent while others produced a maximum amount in 6 per cent ethyl alcohol. Maximum yields of acetic acid in 3 per cent alcohol-containing media reached 86.6 per cent of the theoretical yield with one organism; in 6 per cent reached 68.8 per cent of the theoretical yield with another organism.
5. The difference in maximum average yields for variations in pH with individual organisms was influenced more by the spasmodic behaviour of organisms in acid production than by the change in pH. A medium at an initial pH of 5.0 was generally favourable for acid production.
6. The size of inocula did not affect the rate of acid production by individual organisms.
7. In mixed inocula acetic acid production was not influenced by the effect of each organism alone but by the effect of the mixture.

C. The conversion of ethyl alcohol to acetic acid by *Acetobacter* was determined by the individual nutrient requirements of species.

I. CLAIM TO ORIGINAL RESEARCH

A method to isolate Acetobacter species from Quebec apples was described. The method was different from that successfully used in England.

The production of acetic acid by Acetobacter appears to have received more interest industrially than academically. It seems, however, that difficulties in the industrial production of vinegar are due to the lack of scientific knowledge regarding the physical and chemical factor requirements of Acetobacter for the efficient conversion of ethyl alcohol to acetic acid.

The work reported in this thesis comprises an attempt to supply information, heretofore lacking, about those physical and chemical factors that affect the enzyme system of Acetobacter species in the oxidation of alcohol.

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APPENDIX TABLE I

SPASMODIC ACID PRODUCTION BY ACETOBACTER ORGANISMS

SERIES OF SAMPLE TITRATIONS IN WHICH THE DIFFERENCE BETWEEN THE LARGEST AND SECOND LARGEST OF THREE SIMILAR FERMENTING MEDIA IS NINE ML. OR GREATER

Organism	Condition	Fermenting media	Titration reading of samples, ml.				
			2 day	4 day	6 day	8day	10 day
A. acetosum	pH 7.0	1	1.20	11.20	40.10	37.00	41.50[11]
		2	1.20	10.40	41.60	38.00	36.50
		3	1.80	21.60	41.30	37.80	39.80
A. kützingianum	6 % ethyl alcohol	1	1.00	1.70	3.90	19.10	59.50
		2	1.05	1.40	3.50	17.50	49.20
		3	0.95	1.60	4.20	23.50	63.00
A. kützingianum	pH 4.0	1	2.50	5.70	26.00	36.70	64.60
		2	2.50	15.10	37.80	45.00	74.00
		3	2.50	13.00	36.90	49.00	67.00
A. kützingianum	pH 4.5	1	2.10	15.30	40.20	61.90	59.30
		2	2.10	9.90	31.20	56.40	71.60
		3	1.90	10.60	33.60	58.30	69.20
A. kützingianum	pH 5.0	1	2.10	9.10	22.30	54.60	78.00[11]
		2	2.20	13.00	33.20	58.70	80.20
		3	2.20	12.60	32.30	60.50	81.20
A. rancens	3% ethyl alcohol	1	14.60	21.30	20.80	41.40	24.60[11]
		2	10.00	16.80	36.00	39.20	25.70
		3	10.40	15.30	17.30	29.50	30.00
A. rancens	6% ethyl alcohol	1	1.05	18.70	26.20	60.00	66.40
		2	0.95	17.60	22.40	33.90	53.40
		3	1.15	19.30	31.50	62.90	63.80
A. rancens	pH 4.0	1	2.40	19.50	20.50	32.90	35.00
		2	2.40	21.70	23.40	35.10	61.30
		3	2.80	21.50	21.00	37.60	67.70
A. rancens	pH 5.0	1	1.70	21.40	20.40	62.50	72.70[11]
		2	1.80	16.30	17.30	32.30	71.00
		3	2.40	19.60	18.30	55.60	71.40
A. rancens	pH 5.5	1	10.00	11.80	14.00	23.50	59.50[12]
		2	8.00	11.00	11.60	16.70	50.90
		3	4.30	12.70	12.20	39.00	60.20

continued

11 - Eleven day instead of ten day reading.
12 - Twelve day instead of ten day reading.

APPENDIX TABLE I continued

Organism	Condition	Fermenting Media	Titration reading of samples, ml.				
			2 day	4 day	6 day	8 day	10 day
A. rancens	pH 6.5	1	1.60	19.90	24.70	26.50	47.00
		2	1.50	19.90	23.20	43.80	59.00
		3	1.40	20.20	23.80	39.50	43.80
A. rancens	3 x standard size of inoculum	1	3.10	3.90	4.00	12.50	51.40
		2	3.10	3.80	4.10	4.20	5.40
		3	2.80	3.80	3.80	5.20	9.90
A. rancens	5 x standard size of inoculum	1	3.00	3.70	4.00	12.20	49.70
		2	2.70	3.70	3.90	5.00	6.00
		3	2.90	3.70	3.80	9.60	22.90
A. gluconicum	pH 5.5	1	1.10	1.50	1.50	1.70	2.10[12]
		2	1.10	2.00	18.60	41.46	40.50
		3	1.10	1.30	2.00	2.60	36.60
A. melanogenum 1	6% ethyl alcohol	1	0.80	1.10	14.90	46.30	67.00
		2	0.70	0.80	11.30	44.50	67.00
		3	0.90	1.00	13.10	46.70	46.00
A. oxydans 1	pH 4.5	1	2.20	36.50	48.10	65.10	67.00
		2	2.20	38.70	48.30	63.30	72.10
		3	2.20	27.50	49.00	62.90	68.00
A. oxydans 2	6% ethyl alcohol	1	0.90	2.20	39.60	49.20	52.60
		2	0.95	1.40	34.40	65.20	65.40
		3	0.95	1.40	36.30	62.70	61.90
A. oxydans 2	pH 4.0	1	4.00	45.90	49.90	45.30	56.50
		2	3.20	45.20	48.30	45.80	65.00
		3	3.40	43.20	44.30	42.20	78.90
A. oxydans 2	pH 4.5	1	2.80	45.30	45.70	46.10	60.30
		2	2.40	37.10	41.70	43.60	70.70
		3	2.60	44.20	53.40	51.90	57.50
A. oxydans 2	3 x standard size of inoculum	1	5.30	25.40	40.20	39.40	39.40
		2	4.10	25.20	40.90	42.90	42.30
		3	5.20	27.50	42.00	41.90	64.20

APPENDIX TABLE II

THE EFFECT OF VARYING THE INCUBATION TEMPERATURE OF THE INOCULATED YEAST EXTRACT MEDIUM ON THE AMOUNT OF ACETIC ACID PRODUCED

12° to 24° C

Organism		Readings					
		3 day		5 day		8 day	
		Titration, ml [c]	HAc[d]/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm
A. suboxydans	2T[a]	0.63	0.01	0.73	0.00	0.70	0.01
	C[b]	0.55		0.75		0.55	
A. acetosum	2T	1.08	0.03	1.40	0.05	1.90	0.09
	C	0.60		0.60		0.40	
A. ascendens	2T	0.90	0.02	1.05	0.03	2.88	0.15
	C	0.50		0.55		0.45	
A. pasteurianum	2T	0.98	0.04	1.15	0.05	1.68	0.08
	C	0.40		0.35		0.30	
A. turbidans	2T	0.83	0.02	1.10	0.03	1.35	0.05
	C	0.55		0.55		0.50	
A. kützingianum	2T	0.95	0.02	1.40	0.05	3.40	0.17
	C	0.65		0.55		0.50	
A. aceti 1	2T	1.13	0.04	3.85	0.21	8.55	0.49
	C	0.55		0.30		0.35	
A. aceti 2	2T	1.13	0.04	16.05	0.95	37.48	2.23
	C	0.45		0.15		0.30	
A. rancens	2T	6.85	0.39	11.30	0.65	42.05	2.51
	C	0.40		0.40		0.20	
A. gluconicum	2T	0.73	0.01	0.85	0.01	1.28	0.04
	C	0.55		0.65		0.70	
A. melanogenum 1	2T	1.13	0.03	1.53	0.06	2.08	0.09
	C	0.70		0.60		0.65	
A. oxydans 1	2T	1.03	0.03	2.88	0.15	17.75	1.05
	C	0.60		0.45		0.25	
A. oxydans 2	2T	2.08	0.09	18.78	1.09	44.48	2.65
	C	0.55		0.55		0.35	

a - 2T - Average taken from two tests.
b - C - Control.
c - The amount of N/10 NaOH required to neutralize 10 ml. medium.
d - HAc - Acetic acid.

APPENDIX TABLE III

THE EFFECT OF VARYING THE INCUBATION TEMPERATURE ON THE INOCULATED YEAST EXTRACT MEDIUM ON THE AMOUNT OF ACETIC ACID PRODUCED

25° C

Organism		Readings					
		3 day		5 day		8 day	
		Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm
A. suboxydans	2T	0.78	0.01	0.80	0.01	0.68	0.00
	C	0.70		0.70		0.65	
A. acetosum	2T	2.70	0.13	26.98	1.59	39.63	2.37
	C	0.55		0.48		0.15	
A. ascendens	2T	1.45	0.05	13.65	0.80	41.55	2.48
	C	0.55		0.35		0.15	
A. pasteurianum	2T	0.90	0.02	1.25	0.05	1.75	0.09
	C	0.50		0.35		0.30	
A. turbidans	2T	1.00	0.02	1.07	0.03	1.10[1]	0.05
	C	0.65		0.55		0.25	
A. kützingianum	2T	1.68	0.07	10.53	0.61	42.65	2.55
	C	0.55		0.38		0.20	
A. aceti 1	2T	17.90	1.04	31.68	1.89	44.50	2.67
	C	0.50		0.15		0.00	
A. aceti 2	2T	24.75	1.46	43.27	2.59	31.30	1.88
	C	0.35		0.10		0.00	
A. rancens	2T	18.43	1.08	39.93	2.38	20.95	1.26
	C	0.40		0.25		0.00	
A. gluconicum	2T	0.85[1]	0.02	1.60[1]	0.07	2.30[1]	0.11
	C	0.60		0.50		0.50	
A. melanogenum 1	2T	1.33	0.04	2.18	0.09	3.48	0.17
	C	0.70		0.70		0.65	
A. oxydans 1	2T	5.28	0.28	43.08	2.57	35.53	2.13
	C	0.55		0.25		0.00	
A. oxydans 2	2T	23.43	1.38	43.48	2.59	42.28	2.53
	C	0.45		0.30		0.05	

1 - Average taken from one test .

APPENDIX TABLE IV

THE EFFECT OF VARYING THE INCUBATION TEMPERATURE OF THE INOCULATED YEAST EXTRACT MEDIUM ON THE AMOUNT OF ACETIC ACID PRODUCED

30° C

Organisms		Readings					
		3 day		5 day		8 day	
		Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm
A. suboxydans	2T	0.75	0.01	0.73	0.01	0.78	0.01
	C	0.65		0.60		0.70	
A. acetosum	2T	1.55	0.06	26.55	1.58	38.65	2.30
	C	0.50		0.30		0.35	
A. ascendens	2T	2.03	0.09	23.18	1.38	33.43	1.99
	C	0.55		0.20		0.25	
A. pasteurianum	2T	1.03	0.03	1.25	0.08	1.65	0.10
	C	0.50		0.00		0.00	
A. turbidans	2T	0.85	0.02	0.75	0.02	0.93	0.03
	C	0.50		0.35		0.40	
A. kützingianum	2T	2.78	0.14	14.05	0.83	41.25	2.48
	C	0.50		0.30		0.00	
A. aceti 1	2T	4.75	0.26	38.53	2.31	40.80	2.45
	C	0.45		0.00		0.00	
A. aceti 2	2T	37.10	2.23	37.70	2.26	16.20	0.97
	C	0.00		0.00		0.00	
A. rancens	2T	31.53	1.89	33.00	1.98	22.73	1.36
	C	0.00		0.00		0.00	
A. gluconicum	2T	0.70	0.00	0.95	0.02	1.50	0.06
	C	0.80		0.55		0.55	
A. melanogenum 1	2T	1.48	0.05	2.38	0.11	3.83	0.19
	C	0.65		0.60		0.60	
A. oxydans 1	2T	5.53	0.31	39.95	2.39	29.75	1.79
	C	0.45		0.20		0.00	
A. oxydans 2	2T	21.70	1.28	38.80	2.33	32.45	1.95
	C	0.30		0.00		0.00	

APPENDIX TABLE V

THE EFFECT OF VARYING THE INCUBATION TEMPERATURE OF THE INOCULATED YEAST EXTRACT MEDIUM ON THE AMOUNT OF ACETIC ACID PRODUCED

35° C

Organism		Readings					
		3 day		5 day		8 day	
		Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm
A. suboxydans	2T	0.85	0.01	0.88	0.01	0.95	0.01
	C	0.65		0.75		0.80	
A. acetosum	2T	1.05	0.02	1.25	0.08	5.00	0.30
	C	0.65		0.00		0.00	
A. ascendens	2T	7.30	0.41	19.18	1.14	17.45	1.03
	C	0.50		0.20		0.25	
A. pasteurianum	2T	1.00	0.03	1.20	0.06	1.63	0.08
	C	0.45		0.25		0.35	
A. turbidans	2T	0.73	0.01	1.20[1]	0.04	1.45[1]	0.07
	C	0.50		0.50		0.35	
A. kützingianum	2T	1.65	0.01	2.83	0.15	17.53	1.04
	C	0.45		0.40		0.20	
A. aceti 1	2T	1.25	0.05	2.08	0.11	22.58	1.34
	C	0.35		0.30		0.20	
A. aceti 2	2T	32.40	1.94	34.45	2.07	36.08	2.17
	C	0.00		0.00		0.00	
A. rancens	2T	26.85	1.61	40.25	2.42	39.38	2.35
	C	0.00		0.00		0.20	
A. gluconicum	2T	0.70	0.01	0.73	0.00	0.75	0.01
	C	0.60		0.75		0.60	
A. melanogenum 1	2T	1.35	0.04	1.73	0.07	2.48	0.10
	C	0.70		0.65		0.75	
A. oxydans 1	2T	1.58	0.07	25.80	1.54	32.53	1.94
	C	0.40		0.15		0.20	
A. oxydans 2	2T	1.28	0.06	42.10	2.53	40.35	2.41
	C	0.35		0.00		0.15	

APPENDIX TABLE VI

THE EFFECT OF VARYING THE INCUBATION TEMPERATURE OF THE INOCULATED YEAST EXTRACT MEDIUM ON THE AMOUNT OF ACETIC ACID PRODUCED

MAXIMUM YIELD PRODUCED AND TIME REQUIRED BY EACH ORGANISM FOR EVERY VARIATION IN TEMPERATURE

From Appendix Tables II, III, IV, V

Organism		Temperature, °C			
		12-24	25	30	35
A. suboxydans	HAc/100 ml. medium, gm	0.01	0.01	0.01	0.01
	Time, days	3	3	3	3
A. acetosum	HAc/100 ml. medium, gm	0.09	2.37	2.30	0.30
	Time, days	8	8	8	8
A. ascendens	HAc/100 ml. medium, gm	0.15	2.48	1.99	1.14
	Time, days	8	8	8	5
A. pasteurianum	HAc/100 ml. medium, gm	0.08	0.09	0.10	0.08
	Time, days	8	8	8	8
A. turbidans	HAc/100 ml. medium, gm	0.05	0.05	0.03	0.07
	Time, days	8	8	8	8
A. kützingianum	HAc/100 ml. medium, gm	0.17	2.55	2.48	1.04
	Time, days	8	8	8	8
A. aceti 1	HAc/100 ml. medium, gm	0.49	2.67	2.45	1.34
	Time, days	8	8	8	8
A. aceti 2	HAc/100 ml. medium, gm	2.23	2.59	2.26	2.17
	Time, days	8	5	5	8
A. rancens	HAc/100 ml. medium, gm	2.51	2.38	1.98	2.42
	Time, days	8	5	5	5
A. gluconicum	HAc/100 ml. medium, gm	0.04	0.11	0.06	0.01
	Time, days	8	8	8	3
A. melanogenum 1	HAc/100 ml. medium, gm	0.09	0.17	0.19	0.10
	Time, days	8	8	8	8
A. oxydans 1	HAc/100 ml. medium, gm	1.05	2.57	2.39	1.94
	Time, days	8	5	5	8
A. oxydans 2	HAc/100 ml. medium, gm	2.65	2.59	2.33	2.53
	Time, days	8	5	5	5

═══ Maximum yield of each organism for temperature.

——— Second best yield of each organism for temperature.

APPENDIX TABLE VII

THE EFFECT OF VARYING THE SURFACE AREA OF THE INOCULATED YEAST EXTRACT MEDIUM ON THE AMOUNT OF ACETIC ACID PRODUCED

SURFACE AREA: 43.9 sq. cm. (250 flask)

Organism	Optimum Conditions		Readings					
			3 day		5 day		8 day	
	Temperature, °C		Titration, ml.	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm
A. suboxydans	35	2T	0.85	0.01	0.88	0.01	0.95	0.01
		C	0.65		0.75		0.80	
A. acetosum	25	2T	2.70	0.13	26.98	1.59	39.63	2.37
		C	0.55		0.48		0.15	
A. ascendens	25	2T	1.45	0.05	13.65	0.80	41.55	2.48
		C	0.55		0.35		0.15	
A. pasteurianum	30	2T	1.03	0.03	1.25	0.08	1.65	0.10
		C	0.50		0.00		0.00	
A. turbidans	35	2T	0.73	0.01	1.20[1]	0.04	1.45[1]	0.07
		C	0.50		0.50		0.35	
A. kützingianum	25	2T	1.68	0.07	10.53	0.61	42.65	2.55
		C	0.55		0.38		0.20	
A. aceti 1	25	2T	17.90	1.04	31.68	1.89	44.50	2.67
		C	0.50		0.15		0.00	
A. aceti 2	25	2T	24.75	1.46	43.27	2.59	31.30	1.88
		C	0.35		0.10		0.00	
A. rancens	35	2T	26.85	1.61	40.25	2.42	39.38	2.35
		C	0.00		0.00		0.20	
A. gluconicum	25	2T	0.85[1]	0.02	1.60[1]	0.07	2.30[1]	0.11
		C	0.60		0.50		0.50	
A. melanogenum 1	30	2T	1.48	0.05	2.38	0.11	3.83	0.19
		C	0.65		0.60		0.60	
A. oxydans 1	25	2T	5.28	0.28	43.08	2.57	35.53	2.13
		C	0.55		0.25		0.00	
A. oxydans 2	25	2T	23.43	1.38	43.48	2.59	42.28	2.53
		C	0.45		0.30		0.05	

APPENDIX TABLE VIII

THE EFFECT OF VARYING THE SURFACE AREA OF THE INOCULATED YEAST EXTRACT MEDIUM ON THE AMOUNT OF ACETIC ACID PRODUCED

SURFACE AREA: 76.6 sq. cm. (500 flask)

Organism	Optimum conditions		Readings					
			3 day		5 day		8 day	
	Temperature, °C		Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm
A. suboxydans	35	2T	0.78	0.01	0.80	0.00	0.90	0.04
		C	0.65		0.75		0.20	
A. acetosum	25	2T	4.10	0.22	39.60	2.36	37.20	2.21
		C	0.40		0.30		0.30	
A. ascendens	25	2T	13.05	0.76	21.68	1.29	30.98	1.84
		C	0.35		0.20		0.30	
A. pasteurianum	30	2T	1.40	0.07	1.95	0.10	2.75	0.15
		C	0.25		0.30		0.30	
A. turbidans	35	2T	1.13	0.04	1.28	0.05	1.53	0.08
		C	0.40		0.40		0.25	
A. kützingianum	25	2T	6.90	0.39	22.70	1.35	36.70	2.19
		C	0.35		0.25		0.20	
A. aceti 1	25	2T	38.40	2.30	38.48	2.30	44.55	2.67
		C	0.15		0.20		0.00	
A. aceti 2	25	2T	38.85	2.33	31.05	1.86	5.40	0.32
		C	0.00		0.00		0.00	
A. rancens	35	2T	35.98	2.15	44.43	2.67	43.10	2.59
		C	0.20		0.00		0.00	
A. gluconicum	25	2T	0.93	0.01	1.25	0.03	1.88	0.06
		C	0.70		0.70		0.85	
A. melanogenum 1	30	2T	2.33	0.10	3.40	0.16	4.75	0.24
		C	0.70		0.70		0.70	
A. oxydans 1	25	2T	23.75	1.41	33.28	2.00	26.03	1.56
		C	0.20		0.00		0.00	
A. oxydans 2	25	2T	45.10	2.71	43.93	2.64	43.38	2.60
		C	0.00		0.00		0.00	

APPENDIX TABLE IX

THE EFFECT OF VARYING THE SURFACE AREA OF THE INOCULATED YEAST EXTRACT MEDIUM ON THE AMOUNT OF ACETIC ACID PRODUCED

MAXIMUM YIELD PRODUCED AND TIME REQUIRED BY EACH ORGANISM FOR EVERY VARIATION IN SURFACE AREA

From Appendix Tables VII and VIII

Organism		Surface area, sq. cm.	
		43.9	76.6
A. suboxydans	HAc/100 ml. medium, gm	0.01	0.04
	Time, days	3	8
A. acetosum	HAc/100 ml. medium, gm	2.37	2.36
	Time, days	8	5
A. ascendens	HAc/100 ml. medium, gm	2.48	1.84
	Time, days	8	8
A. pasteurianum	HAc/100 ml. medium, gm	0.10	0.15
	Time, days	8	8
A. turbidans	HAc/100 ml. medium, gm	0.07	0.08
	Time, days	8	8
A. kützingianum	HAc/100 ml. medium, gm	2.55	2.19
	Time, days	8	8
A. aceti 1	HAc/100 ml. medium, gm	2.67	2.67
	Time, days	8	8
A. aceti 2	HAc/100 ml. medium, gm	2.59	2.33
	Time, days	5	3
A. rancens	HAc/100 ml. medium, gm	2.42	2.67
	Time, days	5	5
A. gluconicum	HAc/100 ml. medium, gm	0.11	0.06
	Time, days	8	8
A. melanogenum 1	HAc/100 ml. medium, gm	0.19	0.24
	Time, days	8	8
A. oxydans 1	HAc/100 ml. medium, gm	2.57	2.00
	Time, days	5	5
A. oxydans 2	HAc/100 ml. medium, gm	2.59	2.71
	Time, days	5	3

APPENDIX TABLE X

THE EFFECT OF VARYING THE INITIAL ETHYL ALCOHOL CONTENT OF THE INOCULATED YEAST EXTRACT MEDIUM ON THE AMOUNT OF ACETIC ACID PRODUCED

3 PER CENT ETHYL ALCOHOL

Organism	Optimum conditions		Readings									
			2 day		4 day		6 day		8 day		11 day	
	Temperature, °C		Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm
A. suboxydans	35	3T[e]	0.93	0.02	0.87	0.02	1.03	0.02	1.00	0.02	1.37	0.05
		C	0.65		0.60		0.70		0.75		0.50	
A. acetosum	25	3T	1.53	0.06	7.57	0.44	31.43	1.86	39.80	2.36	32.83	1.94
		C	0.55		0.30		0.45		0.45		0.45	
A. ascendens	25	3T	0.97	0.03	5.57	0.32	15.60	0.91	27.93	1.68	40.80	2.45
		C	0.45		0.30		0.45		0.00		0.00	
A. pasteurianum	30	3T	0.93	0.03	1.00[2]	0.04	1.70	0.08	1.95[2]	0.09	2.37	0.13
		C	0.40		0.40		0.30		0.40		0.25	
A. turbidans	35	3T	0.83	0.02	0.73	0.02	0.90	0.03	1.00	0.03	1.10	0.04
		C	0.50		0.45		0.45		0.45		0.45	
A. kützingianum	25	3T	1.70	0.08	12.88	0.75	25.07	1.50	45.07	2.70	37.10	2.23
		C	0.40		0.40		0.10		0.05		0.00	
A. aceti 1	25	3T	1.23	0.04	15.63	0.92	22.60	1.34	33.53	2.00	44.17	2.64
		C	0.50		0.30		0.20		0.20		0.20	
A. aceti 2	25	3T	3.37	0.18	31.27	1.87	45.33	2.72	41.33	2.48	22.60	1.34
		C	0.30		0.05		0.00		0.00		0.20	
A. rancens	35	3T	11.67	0.67	17.80	1.05	24.70	1.47	36.70	2.18	26.77	1.58
		C	0.50		0.30		0.20		0.30		0.40	
A. gluconicum	25	3T	0.83	0.02	1.20	0.04	2.30	0.09	2.80	0.12	2.90[1]	0.13
		C	0.55		0.60		0.75		0.80		0.75	
A. melanogenum 1	30	3T	0.93	0.02	1.90	0.08	2.90	0.13	3.27	0.16	5.33[2]	0.27
		C	0.65		0.60		0.70		0.60		0.85	
A. oxydans 1	25	3T	1.40	0.06	23.78	1.41	39.30	2.35	43.97	2.63	34.30	2.05
		C	0.40		0.30		0.15		0.20		0.15	
A. oxydans 2	25	3T	2.93	0.15	39.87	2.38	46.27	2.78	43.00	2.58	35.87	2.14
		C	0.50		0.20		0.00		0.00		0.20	

2 - Average taken from two tests.

e - 3T - Average taken from three tests.

APPENDIX TABLE XI

THE EFFECT OF VARYING THE INITIAL ETHYL ALCOHOL CONTENT OF THE INOCULATED YEAST EXTRACT MEDIUM ON THE AMOUNT OF ACETIC ACID PRODUCED

6 PER CENT ETHYL ALCOHOL

Organism	Optimum Conditions		Readings							
			2 day		4 day		6 day		8 day		10 day	
	Temperature, °C		Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm
A. suboxydans	35	3T	0.80	0.00	0.77	0.00	0.83	0.00	0.90	0.01	0.93	0.03
		C	0.80		0.75		0.80		0.80		0.50	
A. acetosum	25	3T	1.12	0.03	1.40	0.06	1.67	0.09	2.23	0.12	2.63	0.14
		C	0.70		0.40		0.20		0.30		0.30	
A. ascendens	25	3T	1.05	0.03	1.27	0.05	1.60	0.07	6.70	0.38	21.87	1.29
		C	0.55		0.50		0.40		0.40		0.30	
A. pasteurianum	30	3T	0.97	0.02	1.23	0.04	1.23	0.04	1.60	0.08	2.00	0.10
		C	0.65		0.50		0.50		0.35		0.40	
A. turbidans	35	3T	0.90	0.01	0.77	0.02	0.73	0.01	0.87	0.03	0.93	0.03
		C	0.70		0.45		0.50		0.45		0.50	
A. kützingianum	25	3T	1.00	0.03	1.57	0.07	3.87	0.23	20.03	1.20	57.23	3.43
		C	0.55		0.45		0.00		0.00		0.15	
A. aceti 1	25	3T	0.97	0.02	1.13	0.05	1.40	0.08	1.70	0.10	1.90	0.11
		C	0.65		0.25		0.00		0.00		0.00	
A. aceti 2	25	3T	1.10	0.05	11.70	0.70	48.30	2.90	69.67	4.18	73.60	4.42
		C	0.30		0.00		0.00		0.00		0.00	
A. rancens	35	3T	1.05	0.03	18.53	1.11	26.70	1.60	52.27	3.13	61.20	3.67
		C	0.55		0.10		0.00		0.10		0.00	
A. gluconicum	25	3T	0.83	0.01	0.70	0.00	0.67	0.00	0.73	0.00	0.83	0.00
		C	0.75		0.80		0.75		0.90		0.95	
A. melanogenum 1	30	3T	0.80	0.00	0.97	0.02	13.10	0.74	45.83	2.71	60.00	3.59
		C	0.75		0.70		0.75		0.60		0.20	
A. oxydans 1	25	3T	1.02	0.03	1.63	0.09	17.80	1.06	55.03	3.30	72.80	4.36
		C	0.60		0.20		0.10		0.00		0.20	
A. oxydans 2	25	3T	0.93	0.02	1.67	0.10	36.77	2.21	59.03	3.54	59.97	3.60
		C	0.55		0.00		0.00		0.00		0.05	

APPENDIX TABLE XII

THE EFFECT OF VARYING THE INITIAL ETHYL ALCOHOL CONTENT OF THE INOCULATED YEAST EXTRACT MEDIUM ON THE AMOUNT OF ACETIC ACID PRODUCED

9 PER CENT ETHYL ALCOHOL

Organism	Optimum conditions		Readings									
			2 day		4 day		6 day		8 day		10 day	
	Temperature, °C		Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm
A. suboxydans	35	3T	0.87	0.01	1.10	0.02	0.90	0.01	0.80	0.00	0.80	0.00
		C	0.75		0.85		0.80		0.85		0.90	
A. acetosum	25	3T	0.93	0.03	1.07	0.04	1.13	0.05	1.33	0.06	1.30	0.07
		C	0.40		0.40		0.25		0.30		0.20	
A. ascendens	25	3T	0.77	0.01	1.07	0.04	0.97	0.03	0.90	0.03	1.23	0.06
		C	0.60		0.40		0.40		0.35		0.30	
A. pasteurianum	30	3T	0.87	0.02	0.90	0.02	0.87	0.02	0.83	0.01	0.90	0.02
		C	0.60		0.50		0.60		0.65		0.65	
A. turbidans	35	3T	0.73	0.01	0.97	0.03	0.73	0.02	0.73	0.02	0.97	0.04
		C	0.50		0.55		0.45		0.45		0.30	
A. kützingianum	25	3T	0.83	0.02	1.20	0.05	1.20	0.06	1.30	0.07	1.63	0.09
		C	0.45		0.30		0.25		0.20		0.10	
A. aceti 1	25	3T	0.70	0.02	0.80	0.03	0.80	0.04	0.77	0.05	0.77	0.05
		C	0.45		0.30		0.15		0.00		0.00	
A. aceti 2	25	3T	0.83	0.04	1.13	0.05	1.30	0.07	1.73	0.10	1.93	0.11
		C	0.15		0.30		0.15		0.10		0.10	
A. rancens	35	3T	0.87	0.04	0.87	0.05	0.80	0.04	0.73	0.04	0.87	0.05
		C	0.20		0.10		0.20		0.00		0.00	
A. gluconicum	25	3T	0.77	0.01	0.87	0.00	0.87	0.00	0.87	0.00	0.87	0.00
		C	0.65		0.85		0.80		0.80		0.80	
A. melanogenum 1	30	3T	0.77	0.00	0.87	0.01	0.83	0.01	0.80	0.00	0.87	0.00
		C	0.70		0.70		0.70		0.75		0.80	
A. oxydans 1	25	3T	0.87	0.03	1.10	0.05	1.33	0.07	1.73	0.09	3.50	0.21
		C	0.45		0.20		0.25		0.20		0.05	
A. oxydans 2	25	3T	0.87	0.04	1.13	0.06	1.33	0.07	16.77	1.01	38.53	2.31
		C	0.15		0.10		0.20		0.00		0.00	

APPENDIX TABLE XIII

A COMPARISON OF EFFICIENCY IN STUDYING THE RATE OF ACETIC ACID PRODUCTION BY USING DIFFERENT SAMPLING-TIME INTERVALS

MAXIMUM YIELD PRODUCED AND TIME REQUIRED BY EACH ORGANISM FOR EVERY VARIATION IN SAMPLING-TIME INTERVALS

From Appendix Tables VII and X

Organism		Time intervals, days	
		3,5,8	2,4,6,8,10
A. suboxydans	HAc/100 ml. medium, gm	0.01	0.05
	Time, days	3	11
A. acetosum	HAc/100 ml. medium, gm	2.37	2.36
	Time, days	8	8
A. ascendens	HAc/100 ml. medium, gm	2.48	2.45
	Time, days	8	11
A. pasteurianum	HAc/100 ml. medium, gm	0.10	0.13
	Time, days	8	11
A. turbidans	HAc/100 ml. medium, gm	0.07	0.04
	Time, days	8	11
A. kützingianum	HAc/100 ml. medium, gm	2.55	2.70
	Time, days	8	8
A. aceti 1	HAc/100 ml. medium, gm	2.67	2.64
	Time, days	8	11
A. aceti 2	HAc/100 ml. medium, gm	2.59	2.72
	Time, days	5	6
A. rancens	HAc/100 ml. medium, gm	2.42	2.18
	Time, days	5	8
A. gluconicum	HAc/100 ml. medium, gm	0.11	0.13
	Time, days	8	11
A. melanogenum 1	HAc/100 ml. medium, gm	0.19	0.27
	Time, days	8	11
A. oxydans 1	HAc/100 ml. medium, gm	2.57	2.63
	Time, days	5	8
A. oxydans 2	HAc/100 ml. medium, gm	2.59	2.78
	Time, days	5	6

APPENDIX TABLE XIV

THE EFFECT OF VARYING THE INITIAL ETHYL ALCOHOL CONTENT OF THE INOCULATED YEAST EXTRACT MEDIUM ON THE AMOUNT OF ACETIC ACID PRODUCED

MAXIMUM YIELD PRODUCED AND TIME REQUIRED BY EACH ORGANISM FOR EVERY VARIATION IN ETHYL ALCOHOL CONCENTRATION

From Appendix Tables X, XI, XII

Organism		Ethyl alcohol, per cent		
		3	6	9
A. suboxydans	HAc/100 ml. medium, gm	0.05	0.03	0.02
	Time, days	11	10	4
A. acetosum	HAc/100 ml. medium, gm	2.36	0.14	0.07
	Time, days	8	10	10
A. ascendens	HAc/100 ml. medium, gm	2.45	1.29	0.06
	Time, days	11	10	10
A. pasteurianum	HAc/100 ml. medium, gm	0.13	0.10	0.02
	Time, days	11	10	2
A. turbidans	HAc/100 ml. medium, gm	0.04	0.03	0.04
	Time, days	11	8	10
A. kützingianum	HAc/100 ml. medium, gm	2.70	3.43	0.09
	Time, days	8	10	10
A. aceti 1	HAc/100 ml. medium, gm	2.64	0.11	0.05
	Time, days	11	10	8
A. aceti 2	HAc/100 ml. medium, gm	2.72	4.42	0.11
	Time, days	6	10	10
A. rancens	HAc/100 ml. medium, gm	2.18	3.67	0.05
	Time, days	8	10	4
A. gluconicum	HAc/100 ml. medium, gm	0.13	0.01	0.01
	Time, days	11	2	2
A. melanogenum 1	HAc/100 ml. medium, gm	0.27	3.59	0.01
	Time, days	11	10	4
A. oxydans 1	HAc/100 ml. medium, gm	2.63	4.36	0.21
	Time, days	8	10	10
A. oxydans 2	HAc/100 ml. medium, gm	2.78	3.60	2.31
	Time, days	6	10	10

APPENDIX TABLE XV

THE EFFECT OF VARYING THE INITIAL pH OF THE INOCULATED YEAST EXTRACT MEDIUM ON THE AMOUNT OF ACETIC ACID PRODUCED

pH 4.0

Organism	Optimum conditions			Readings									
				2 day		4 day		6 day		8 day		10 day	
	Temperature, °C	Ethyl alcohol %		Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm
A. suboxydans	35	3	3T	2.17	0.00	2.17	0.00	2.37	0.00	2.17	0.00	2.43	0.01
			C	2.20		2.30		2.35		2.25		2.25	
A. acetosum	25	3	3T	3.57	0.11	31.50	1.80	39.60	2.29	36.20	2.09	34.10	1.97
			C	1.70		1.55		1.40		1.45		1.30	
A. ascendens	25	3	3T	4.93	0.19	26.17	1.50	32.03	1.86	34.93	2.02	38.07	2.22
			C	1.75		1.15		1.10		1.20		1.15	
A. pasteurianum	30	3	3T	2.40	0.02	2.60	0.04	3.07	0.08	3.23	0.09	3.40	0.11
			C	2.10		1.90		1.80		1.80		1.60	
A. turbidans	35	9	3T	2.17	0.01	2.23	0.01	2.30	0.02	2.33	0.03	2.23	0.04
			C	2.00		2.00		1.90		1.80		1.60	
A. kützingianum	25	6	3T	2.50	0.05	11.27	0.62	33.57	1.97	43.57	2.57	68.53	4.07
			C	1.75		0.90		0.70		0.70		0.65	
A. aceti 1	25	3	3T	3.73	0.11	29.10	1.68	41.60	2.47	41.40	2.46	44.77	2.67
			C	1.85		1.10		0.45		0.40		0.30	
A. aceti 2	25	6	3T	2.60	0.07	18.73	1.06	50.03	2.94	58.50	3.44	66.03	3.90
			C	1.40		1.10		1.00		1.10		1.05	
A. rancens	35	6	3T	2.53	0.12	20.90	1.23	21.63	1.27	35.20	2.08	54.67	3.26
			C	0.60		0.45		0.45		0.55		0.35	
A. gluconicum	25	3	3T	2.20	0.00	2.33	0.01	2.47	0.01	2.93	0.04	3.07	0.05
			C	2.25		2.20		2.25		2.25		2.30	
A. melanogenum 1	30	6	3T	2.57	0.02	2.90	0.04	3.30	0.06	3.80	0.08	4.10	0.10
			C	2.20		2.30		2.35		2.50		2.40	
A. oxydans 1	25	6	3T	2.87	0.09	37.10	2.20	50.20	2.98	52.93	3.15	61.37	3.65
			C	1.45		0.45		0.50		0.40		0.50	
A. oxydans 2	25	6	3T	3.53	0.13	44.77	2.64	47.50	2.83	44.43	2.65	66.80	3.99
			C	1.40		0.70		0.35		0.35		0.35	

APPENDIX TABLE XVI

THE EFFECT OF VARYING THE INITIAL pH OF THE INOCULATED YEAST EXTRACT MEDIUM ON THE AMOUNT OF ACETIC ACID PRODUCED
pH 4.5

Organism	Optimum conditions			Readings									
				2 day		4 day		6 day		8 day		10 day	
	Temperature, °C	Ethyl alcohol %		Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm
A. suboxydans	35	3	3T	1.80	0.01	1.70	0.00	1.83	0.01	1.73	0.01	1.90	0.02
			C	1.70		1.65		1.65		1.65		1.65	
A. acetosum	25	3	3T	2.90	0.09	32.33	1.88	36.50	2.14	35.10	2.06	34.50	2.03
			C	1.40		0.95		0.80		0.85		0.65	
A. ascendens	25	3	3T	2.83	0.09	27.13	1.59	33.13	1.95	36.37	2.15	38.97	2.30
			C	1.35		0.65		0.60		0.60		0.70	
A. pasteurianum	30	3	3T	2.07	0.04	2.03	0.04	2.50	0.08	2.70	0.09	2.80	0.10
			C	1.40		1.30		1.20		1.15		1.10	
A. turbidans	35	9	3T	1.83	0.02	1.80	0.02	1.87	0.03	1.67	0.03	1.73	0.04
			C	1.50		1.45		1.30		1.25		1.10	
A. kützingianum	25	6	3T	2.03	0.03	11.93	0.67	35.00	2.07	58.87	3.50	66.70	3.97
			C	1.55		0.70		0.50		0.55		0.50	
A. aceti 1	25	3	3T	2.23	0.10	32.30[2]	1.93	37.83	2.26	39.55[2]	2.36	42.10	2.51
			C	1.50		0.20		0.20		0.20		0.25	
A. aceti 2	25	6	3T	2.17	0.07	29.50	1.74	59.47	3.54	62.00	3.69	58.63	3.49
			C	1.00		0.50		0.50		0.45		0.40	
A. rancens	35	6	3T	2.07	0.07	21.37	1.26	23.93	1.41	53.57	3.19	66.43	3.96
			C	0.85		0.40		0.45		0.35		0.40	
A. gluconicum	25	3	3T	1.80	0.01	1.80	0.01	2.13	0.03	2.27	0.03	2.53	0.06
			C	1.60		1.60		1.60		1.70		1.60	
A. melanogenum 1	30	6	3T	2.17	0.03	2.60	0.06	2.80	0.06	3.07	0.08	3.37	0.09
			C	1.75		1.65		1.75		1.75		1.90	
A. oxydans 1	25	6	3T	2.20	0.06	34.23	2.03	48.47	2.88	63.77	3.80	69.03	4.13
			C	1.20		0.45		0.45		0.40		0.25	
A. oxydans 2	25	6	3T	2.60	0.08	42.20	2.52	46.93	2.80	47.20	2.82	62.83	3.76
			C	1.25		0.15		0.20		0.25		0.25	

APPENDIX TABLE XVII

THE EFFECT OF VARYING THE INITIAL pH OF THE INOCULATED YEAST EXTRACT MEDIUM ON THE AMOUNT OF ACETIC ACID PRODUCED

pH 5.0

Organism	Optimum conditions			Readings									
				2 day		4 day		6 day		8 day		11 day	
	Temperature, °C	Ethyl alcohol %		Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm
A. suboxydans	35	3	3T	1.30	0.00	1.27	0.00	1.43	0.01	1.47	0.01	1.77	0.02
			C	1.30		1.20		1.35		1.30		1.45	
A. acetosum	25	3	3T	2.20	0.07	23.10	1.35	40.20	2.38	36.77	2.18	34.30	2.03
			C	1.05		0.60		0.50		0.40		0.45	
A. ascendens	25	3	3T	3.30	0.15	18.13	1.06	29.73	1.76	34.43	2.05	39.83	2.37
			C	0.80		0.40		0.40		0.35		0.35	
A. pasteurianum	30	3	3T	1.73	0.05	1.83	0.07	2.17	0.09	2.30	0.10	2.67	0.13
			C	0.95		0.75		0.70		0.60		0.55	
A. turbidans	35	9	3T	1.30	0.02	1.30	0.02	1.23	0.03	1.30	0.04	1.30	0.03
			C	1.00		0.95		0.70		0.70		0.75	
A. kützingianum	25	6	3T	2.17	0.09	11.57	0.67	29.27	1.73	57.93	3.45	79.80	4.77
			C	0.75		0.45		0.50		0.40		0.35	
A. aceti 1	25	3	3T	6.70	0.36	28.10	1.68	37.87	2.27	43.63	2.62	40.90	2.44
			C	0.65		0.15		0.00		0.00		0.20	
A. aceti 2	25	6	3T	3.03	0.16	28.90	1.72	58.40	3.48	66.37	3.97	70.20	4.19
			C	0.40		0.30		0.35		0.15		0.30	
A. rancens	35	6	3T	1.97	0.09	19.10	1.12	18.67	1.10	50.13	3.00	71.70	4.28
			C	0.40		0.40		0.40		0.20		0.40	
A. gluconicum	25	3	3T	1.20	0.00	1.70	0.03	1.63	0.02	1.83	0.03	2.23	0.06
			C	1.20		1.25		1.30		1.30		1.30	
A. melanogenum 1	30	6	3T	1.77	0.03	2.20	0.06	2.30	0.06	2.70	0.08	3.20	0.12
			C	1.25		1.25		1.25		1.30		1.25	
A. oxydans 1	25	6	3T	4.50	0.23	30.47	1.80	48.50	2.89	63.40	3.79	79.80	4.77
			C	0.60		0.40		0.30		0.30		0.30	
A. oxydans 2	25	6	3T	13.73	0.80	49.40	2.95	51.07	3.06	50.60	3.02	79.47	4.77
			C	0.35		0.30		0.15		0.20		0.00	

APPENDIX TABLE XVIII

THE EFFECT OF VARYING THE INITIAL pH OF THE INOCULATED YEAST EXTRACT MEDIUM ON THE AMOUNT OF ACETIC ACID PRODUCED

pH 5.5

Organism	Optimum conditions			Readings									
				2 day		4 day		6 day		8 day		12 day	
	Temperature, °C	Ethyl alcohol %		Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm
A. suboxydans	35	3	3T	1.00	0.00	1.23	0.01	1.27	0.01	1.27	0.02	1.53	0.02
			C	1.00		1.05		1.10		1.00		1.20	
A. acetosum	25	3	3T	2.03	0.77	17.67	1.03	41.20	2.45	37.63	2.23	35.27	2.09
			C	0.75		0.55		0.40		0.40		0.45	
A. ascendens	25	3	3T	2.33	0.10	13.83	0.81	31.20	1.86	36.83	2.20	39.17	2.33
			C	0.65		0.40		0.25		0.25		0.40	
A. pasteurianum	30	3	3T	1.40	0.04	1.73	0.07	2.03	0.10	2.23	0.11	2.77	0.14
			C	0.70		0.60		0.40		0.40		0.40	
A. turbidans	35	9	3T	1.07	0.01	1.40	0.03	1.13	0.02	1.30	0.03	1.30	0.04
			C	0.95		0.85		0.75		0.80		0.60	
A. kützingianum	25	6	3T	1.60	0.07	4.77	0.26	18.40	1.09	41.33	2.46	81.60	4.87
			C	0.40		0.40		0.20		0.30		0.45	
A. aceti 1	25	3	3T	11.60	0.66	33.53	2.01	43.07	2.58	44.33	2.66	39.17	2.35
			C	0.55		0.00		0.00		0.00		0.00	
A. aceti 2	25	6	3T	1.87	0.10	9.57	0.57	43.70	2.61	61.47	3.68	82.67	4.95
			C	0.20		0.15		0.20		0.15		0.20	
A. rancens	35	6	3T	7.43	0.42	11.83	0.69	12.60	0.74	26.40	1.57	56.87	3.39
			C	0.45		0.35		0.20		0.20		0.30	
A. gluconicum	25	3	3T	1.07	0.01	1.60	0.05	7.37	0.41	15.25	0.92	26.40	1.58
			C	0.90		0.85		0.60		0.00		0.00	
A. melanogenum 1	30	6	3T	1.63	0.05	2.00	0.06	2.33	0.08	2.73	0.10	3.47	0.14
			C	0.85		1.00		1.00		1.10		1.15	
A. oxydans 1	25	6	3T	2.00	0.08	22.23	1.31	49.53	2.96	56.20	3.36	74.20	4.43
			C	0.65		0.35		0.25		0.25		0.35	
A. oxydans 2	25	6	3T	4.93	0.26	38.63	2.30	47.43	2.84	49.07	2.94	77.03	4.61
			C	0.65		0.25		0.10		0.15		0.20	

APPENDIX TABLE XIX

THE EFFECT OF VARYING THE INITIAL pH OF THE INOCULATED YEAST EXTRACT MEDIUM ON THE AMOUNT OF ACETIC ACID PRODUCED

pH 6.0

Organism	Optimum conditions			Readings									
				2 day		4 day		6 day		8 day		10 day	
	Temperature, °C	Ethyl alcohol %		Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm
A. suboxydans	35	3	3T C	0.93 0.65	0.02	0.87 0.60	0.02	1.03 0.70	0.02	1.00 0.75	0.02	1.37 0.50	0.05[11]
A. acetosum	25	3	3T C	1.53 0.55	0.06	7.57 0.30	0.44	31.43 0.45	1.86	39.80 0.45	2.36	32.83 0.45	1.94[11]
A. ascendens	25	3	3T C	0.97 0.45	0.03	5.57 0.30	0.32	15.60 0.45	0.91	27.93 0.00	1.68	40.80 0.00	2.45[11]
A. pasteurianum	30	3	3T C	0.93 0.40	0.03	1.00 0.40	0.04	1.70 0.30	0.08	1.95[2] 0.40	0.09	2.37 0.25	0.13[11]
A. turbidans	35	9	3T C	0.73 0.50	0.01	0.97 0.55	0.03	0.73 0.45	0.02	0.73 0.45	0.02	0.97 0.30	0.04
A. kützingianum	25	6	3T C	1.00 0.55	0.03	1.57 0.45	0.07	3.87 0.00	0.23	20.03 0.00	1.20	57.23 0.15	3.43
A. aceti 1	25	3	3T C	1.23 0.50	0.04	15.63 0.30	0.92	22.60 0.20	1.34	33.53 0.20	2.00	44.17 0.20	2.64[11]
A. aceti 2	25	6	3T C	1.10 0.30	0.05	11.70 0.00	0.70	48.30 0.00	2.90	69.67 0.00	4.18	73.60 0.00	4.42
A. rancens	35	6	3T C	1.05 0.55	0.03	18.53 0.10	1.11	26.70 0.00	1.60	52.27 0.10	3.13	61.20 0.00	3.67
A. gluconicum	25	3	3T C	0.83 0.55	0.02	1.20 0.60	0.04	2.30 0.75	0.09	2.80 0.80	0.12	2.90[1] 0.75	0.13[11]
A. melanogenum 1	30	6	3T C	0.80 0.75	0.00	0.97 0.70	0.02	13.10 0.75	0.74	45.83 0.60	2.71	60.00 0.20	3.59
A. oxydans 1	25	6	3T C	1.02 0.60	0.03	1.63 0.20	0.09	17.80 0.10	1.06	55.03 0.00	3.30	72.80 0.20	4.36
A. oxydans 2	25	6	3T C	0.93 0.55	0.02	1.67 0.00	0.10	36.77 0.00	2.21	59.03 0.00	3.54	59.97 0.05	3.60

APPENDIX TABLE XX

THE EFFECT OF VARYING THE INITIAL pH OF THE INOCULATED YEAST EXTRACT MEDIUM ON THE AMOUNT OF ACETIC ACID PRODUCED

pH 6.5

Organism	Optimum conditions			Readings									
				2 day		4 day		6 day		8 day		10 day	
	Temperature, °C	Ethyl alcohol %		Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm
A. suboxydans	35	3	3T	1.03	0.02	1.00	0.01	0.80	0.00	0.97	0.01	1.07	0.04
			C	0.75		0.80		0.75		0.80		0.45	
A. acetosum	25	3	3T	1.43	0.06	16.87	0.99	40.53	2.41	38.00	2.26	33.87	2.01
			C	0.50		0.45		0.30		0.35		0.45	
A. ascendens	25	3	3T	1.43	0.07	9.65[2]	0.55	28.13	1.67	42.17	2.52	41.63	2.47
			C	0.35		0.45		0.25		0.25		0.50	
A. pasteurianum	30	3	3T	1.23	0.04	1.40	0.06	1.47	0.06	1.87	0.09	1.93	0.09
			C	0.50		0.35		0.45		0.45		0.50	
A. turbidans	35	9	3T	0.83	0.02	0.80	0.02	0.83	0.02	0.93	0.03	0.97	0.03
			C	0.55		0.50		0.55		0.50		0.50	
A. kützingianum	25	6	3T	1.97	0.09	11.67	0.69	32.47	1.93	68.93	4.12	71.43	4.27
			C	0.50		0.25		0.30		0.20		0.25	
A. aceti 1	25	3	3T	1.23	0.05	29.17	1.75	35.53	2.13	44.40	2.66	42.97	2.56
			C	0.35		0.00		0.00		0.00		0.30	
A. aceti 2	25	6	3T	1.27	0.07	15.13	0.91	34.80	2.09	64.97	3.90	70.20	4.20
			C	0.15		0.00		0.00		0.00		0.15	
A. rancens	35	6	3T	1.50	0.07	20.00	1.19	23.90	1.42	36.60	2.18	49.93	2.97
			C	0.40		0.20		0.20		0.20		0.40	
A. gluconicum	25	3	3T	0.93	0.02	1.00	0.03	1.43	0.06	1.93	0.10	2.23	0.13
			C	0.65		0.55		0.50		0.25		0.00	
A. melanogenum 1	30	6	3T	1.33	0.04	1.57	0.06	1.83	0.08	2.20	0.12	2.43	0.13
			C	0.70		0.55		0.45		0.25		0.35	
A. oxydans 1	25	6	3T	1.50	0.07	27.03	1.61	42.17	2.52	66.60	3.98	76.93	4.60
			C	0.40		0.20		0.25		0.20		0.35	
A. oxydans 2	25	6	3T	1.20	0.04	34.07	2.04	39.93	2.40	46.23	2.77	58.77	3.53
			C	0.50		0.00		0.00		0.00		0.00	

APPENDIX TABLE XXI

THE EFFECT OF VARYING THE INITIAL pH OF THE INOCULATED YEAST EXTRACT MEDIUM ON THE AMOUNT OF ACETIC ACID PRODUCED

pH 7.0

Organism	Optimum conditions			Readings									
				2 day		4 day		6 day		8 day		11 day	
	Temperature, °C	Ethyl alcohol %		Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm
A. suboxydans	35	3	3T C	0.70 0.50	0.01	0.87 0.60	0.02	0.87 0.65	0.01	1.10[2] 0.65	0.03	1.25[2] 0.65	0.04
A. acetosum	25	3	3T C	1.40 0.50	0.05	14.40 0.35	0.84	41.00 0.30	2.44	37.60 0.20	2.24	39.27 0.00	2.36
A. ascendens	25	3	3T C	0.87 0.45	0.03	3.25[2] 0.40	0.17	18.73 0.30	1.11	38.03 0.30	2.26	34.03 0.15	2.03
A. pasteurianum	30	3	3T C	0.87 0.50	0.02	1.13 0.40	0.04	1.33 0.20	0.07	1.63 0.25	0.08	2.07 0.10	0.12
A. turbidans	35	9	3T C	0.70 0.55	0.01	0.70 0.40	0.02	0.70 0.00	0.04	0.67 0.00	0.04	0.67 0.00	0.04
A. kützingianum	25	6	3T C	1.03 0.45	0.04	4.43 0.50	0.24	24.10 0.25	1.43	58.47 0.25	3.49	74.37 0.15	4.45
A. aceti 1	25	3	3T C	1.10 0.40	0.04	21.53 0.20	1.28	44.23 0.00	2.65	39.90 0.00	2.39	41.23 0.00	2.47
A. aceti 2	25	6	3T C	0.97 0.30	0.04	2.10[2] 0.10	0.12	12.87 0.00	0.77	42.53 0.00	2.55	81.27 0.00	4.88
A. rancens	35	6	3T C	1.00 0.45	0.03	13.87 0.20	0.82	17.57 0.00	1.05	22.53 0.00	1.35	66.00 0.00	3.96
A. gluconicum	25	3	3T C	0.67 0.50	0.01	0.73 0.65	0.01	1.10 0.65	0.03	2.00 0.70	0.08	2.27 0.55	0.10
A. melanogenum 1	30	6	3T C	0.70 0.50	0.01	0.83 0.70	0.01	0.93 0.60	0.02	1.33 0.60	0.04	2.03 0.10	0.12
A. oxydans 1	25	6	3T C	0.93 0.45	0.03	6.23 0.35	0.35	41.00 0.25	2.45	61.50 0.10	3.68	80.37 0.00	4.82
A. oxydans 2	25	6	3T C	1.00[2] 0.50	0.03	24.70 0.00	1.48	50.17 0.00	3.01	57.80 0.00	3.47	77.57 0.00	4.65

APPENDIX TABLE XXII

THE EFFECT OF VARYING THE INITIAL pH OF THE INOCULATED YEAST EXTRACT MEDIUM ON THE AMOUNT OF ACETIC ACID PRODUCED

MAXIMUM YIELD PRODUCED AND TIME REQUIRED BY EACH ORGANISM FOR EVERY VARIATION IN pH

From Appendix Tables XV - XXI

Organism		pH						
		4.0	4.5	5.0	5.5	6.0	6.5	7.0
A. suboxydans	HAc/100 ml. medium, gm Time, days	0.01 10	0.02 10	0.02 11	0.02 8	0.05 11	0.04 10	0.04 11
A. acetosum	HAc/100 ml. medium, gm Time, days	2.29 6	2.14 6	2.38 6	2.45 6	2.36 8	2.41 6	2.44 6
A. ascendens	HAc/100 ml. medium, gm Time, days	2.22 10	2.30 10	2.37 11	2.33 12	2.45 11	2.52[f] 8	2.26 8
A. pasteurianum	HAc/100 ml. medium, gm Time, days	0.11 10	0.10 10	0.13 11	0.14 12	0.13 11	0.09 8	0.12 11
A. turbidans	HAc/100 ml. medium, gm Time, days	0.04 10	0.04 10	0.04 8	0.04 12	0.04 10	0.03 8	0.04 6
A. kützingianum	HAc/100 ml. medium, gm Time, days	4.07 10	3.97 10	4.77 11	4.87 12	3.43 10	4.27 10	4.45 11
A. aceti 1	HAc/100 ml. medium, gm Time, days	2.67 10	2.51 10	2.62 8	2.66 8	2.64 11	2.66 8	2.65 6
A. aceti 2	HAc/100 ml. medium, gm Time, days	3.90 10	3.69 8	4.19 11	4.95 12	4.42 10	4.20 10	4.88 11
A. rancens	HAc/100 ml. medium, gm Time, days	3.26 10	3.96 10	4.28 11	3.39 12	3.67 10	2.97 10	3.96 11
A. gluconicum	HAc/100 ml. medium, gm Time, days	0.05 10	0.06 10	0.06 11	1.58 12	0.13 11	0.13 10	0.10 11
A. melanogenum 1	HAc/100 ml. medium, gm Time, days	0.10 10	0.09 10	0.12 11	0.14 12	3.59 10	0.13 10	0.12 11
A. oxydans 1	HAc/100 ml. medium, gm Time, days	3.65 10	4.13 10	4.77 11	4.43 12	4.36 10	4.60 10	4.82 11
A. oxydans 2	HAc/100 ml. medium, gm Time, days	3.99 10	3.76 10	4.77 11	4.61 12	3.60 10	3.53 10	4.65 11

f - Arrow points to pH used in succeeding experiments.

APPENDIX TABLE XXIII

THE EFFECT OF VARYING THE SIZE OF THE INOCULUM IN YEAST EXTRACT MEDIUM ON THE AMOUNT OF ACETIC ACID PRODUCED

STANDARD SIZE

Organism	Optimum conditions				Readings									
					2 day		4 day		6 day		8 day		10 day	
	Temperature, °C	Ethyl alcohol %	pH		Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm
A. suboxydans	35	3	6.0	3T	0.93	0.02	0.87	0.02	1.03	0.02	1.00	0.02	1.37	0.05[11]
				C	0.65		0.60		0.70		0.75		0.50	
A. acetosum	25	3	5.5	3T	2.03	0.77	17.67	1.03	41.20	2.45	37.63	2.23	35.27	2.09[12]
				C	0.75		0.55		0.40		0.40		0.45	
A. ascendens	25	3	6.0	3T	0.97	0.03	5.57	0.32	15.60	0.91	27.93	1.68	40.80	2.45[11]
				C	0.45		0.30		0.45		0.00		0.00	
A. pasteurianum	30	3	5.5	3T	1.40	0.04	1.73	0.07	2.03	0.10	2.23	0.11	2.77	0.14[12]
				C	0.70		0.60		0.40		0.40		0.40	
A. turbidans	35	9	5.5	3T	1.07	0.01	1.40	0.03	1.13	0.02	1.30	0.03	1.30	0.04[12]
				C	0.95		0.85		0.75		0.80		0.60	
A. kützingianum	25	6	5.5	3T	1.60	0.07	4.77	0.26	18.40	1.09	41.33	2.46	81.60	4.87[12]
				C	0.40		0.40		0.20		0.30		0.45	
A. aceti 1	25	3	5.5	3T	11.60	0.66	33.53	2.01	43.07	2.58	44.33	2.66	39.17	2.35[12]
				C	0.55		0.00		0.00		0.00		0.00	
A. aceti 2	25	6	5.5	3T	1.87	0.10	9.57	0.57	43.70	2.61	61.47	3.68	82.67	4.95[12]
				C	0.20		0.15		0.20		0.15		0.20	
A. rancens	35	6	5.0	3T	1.97	0.09	19.10	1.12	18.67	1.10	50.13	3.00	71.70	4.28[11]
				C	0.40		0.40		0.40		0.20		0.40	
A. gluconicum	25	3	5.5	3T	1.07	0.01	1.60	0.05	7.37	0.41	15.25	0.92	26.40	1.58[12]
				C	0.90		0.85		0.60		0.00		0.00	
A. melanogenum1	30	6	6.0	3T	0.80	0.00	0.97	0.02	13.10	0.74	45.83	2.71	60.00	3.59
				C	0.75		0.70		0.75		0.60		0.20	
A. oxydans 1	25	6	5.0	3T	4.50	0.23	30.47	1.80	48.50	2.89	63.40	3.79	79.80	4.77[11]
				C	0.60		0.40		0.30		0.30		0.30	
A. oxydans 2	25	6	5.0	3T	13.73	0.80	49.40	2.95	51.07	3.06	50.60	3.02	79.47	4.77[11]
				C	0.35		0.30		0.15		0.20		0.00	

APPENDIX TABLE XXIV

THE EFFECT OF VARYING THE SIZE OF THE INOCULUM IN YEAST EXTRACT MEDIUM ON THE AMOUNT OF ACETIC ACID PRODUCED

THREE TIMES STANDARD SIZE

Organism	Optimum conditions				Readings									
					2 day		4 day		6 day		8 day		10 day	
	Temperature, °C	Ethyl alcohol %	pH		Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm
A. suboxydans	35	3	6.0	3T	1.50	0.03	1.67	0.03	1.87	0.05	2.00	0.05	2.13	0.08
				C	1.05		1.15		1.10		1.10		0.80	
A. acetosum	25	3	5.5	3T	2.53	0.09	16.03	0.92	41.03	2.42	41.70	2.48	37.70	2.24
				C	1.00		0.75		0.75		0.40		0.45	
A. ascendens	25	3	6.0	3T	1.50	0.04	2.40	0.09	12.70	0.72	27.07	1.59	33.53	1.97
				C	0.85		0.85		0.75		0.65		0.65	
A. pasteurianum	30	3	5.5	3T	1.60	0.04	1.97	0.06	2.17	0.08	2.33	0.10	2.57	0.11
				C	1.00		0.90		0.85		0.70		0.70	
A. turbidans	35	9	5.5	3T	1.63	0.03	1.83	0.05	2.03	0.06	2.00	0.06	2.00	0.05
				C	1.20		1.05		1.10		1.05		1.20	
A. kützingianum	25	6	5.5	3T	1.93	0.06	2.00	0.08	2.83	0.14	11.03	0.63	28.00	1.65
				C	0.95		0.65		0.45		0.50		0.50	
A. aceti 1	25	3	5.5	3T	1.73	0.04	5.73	0.30	21.83	1.27	29.10	1.73	41.47	2.47
				C	1.00		0.70		0.65		0.30		0.25	
A. aceti 2	25	6	5.5	3T	1.87	0.08	2.80	0.14	9.90	0.57	42.13	2.50	55.07	3.28
				C	0.60		0.40		0.40		0.50		0.40	
A. rancens	25	6	5.0	3T	3.00	0.12	3.83	0.18	3.97	0.19	7.30	0.38	22.23	1.28
				C	0.95		0.80		0.80		0.90		0.95	
A. gluconicum	25	3	5.5	3T	1.40	0.01	1.67	0.03	1.93	0.04	2.23	0.05	2.40	0.05
				C	1.20		1.20		1.30		1.35		1.50	
A. melanogenum 1	30	6	6.0	3T	1.60	0.03	2.00	0.05	2.33	0.07	2.75[2]	0.10	3.13	0.11
				C	1.10		1.10		1.25		1.15		1.30	
A. oxydans 1	25	6	5.0	3T	2.80	0.11	8.57	0.47	14.53	0.83	31.43	1.86	40.77	2.42
				C	0.90		0.70		0.70		0.45		0.50	
A. oxydans 2	25	6	5.0	3T	4.87	0.24	26.03	1.52	41.03	2.43	41.40	2.45	48.63	2.90
				C	0.90		0.65		0.60		0.60		0.35	

THE EFFECT OF VARYING THE SIZE OF THE INOCULUM IN YEAST EXTRACT MEDIUM ON THE AMOUNT OF ACETIC ACID PRODUCED

FIVE TIMES STANDARD SIZE

Organism	Optimum conditions				Readings									
					2 day		4 day		6 day		8 day		10 day	
	Temperature, °C	Ethyl alcohol %	pH		Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm
A. suboxydans	35	3	6.0	3T	1.40	0.02	1.80	0.04	1.67	0.04	1.87	0.05	1.90	0.04
				C	1.10		1.10		1.05		1.10		1.20	
A. acetosum	25	3	5.5	3T	3.47	0.15	27.77	1.62	43.40	2.56	41.93	2.48	38.23	2.26
				C	1.00		0.85		0.80		0.60		0.60	
A. ascendens	25	3	6.0	3T	1.60	0.05	3.67	0.17	18.83	1.08	29.60	1.73	35.30	2.08
				C	0.85		0.80		0.80		0.70		0.70	
A. pasteurianum	30	3	5.5	3T	1.53	0.03	2.07	0.08	2.07	0.08	2.30	0.10	2.63	0.11
				C	1.00		0.80		0.70		0.60		0.75	
A. turbidans	35	9	5.5	3T	1.73	0.04	2.10	0.06	2.00	0.06	2.00	0.07	1.97	0.06
				C	1.15		1.10		1.00		0.90		0.90	
A. kützingianum	25	6	5.5	3T	2.00	0.06	3.50	0.17	4.90	0.26	8.43	0.48	27.03	1.60
				C	1.00		0.60		0.50		0.40		0.30	
A. aceti 1	25	3	5.5	3T	1.83	0.05	13.97	0.80	21.80	1.28	30.07	1.78	45.77	2.73
				C	1.00		0.60		0.40		0.40		0.30	
A. aceti 2	25	6	5.5	3T	1.87	0.08	2.43	0.12	4.60	0.25	34.47	2.03	52.43	3.13
				C	0.60		0.40		0.50		0.60		0.35	
A. rancens	35	6	5.0	3T	2.87	0.12	3.70	0.17	3.90	0.19	8.93	0.49	26.20	1.54
				C	0.95		0.85		0.80		0.85		0.60	
A. gluconicum	25	3	5.5	3T	1.47	0.02	1.73	0.02	1.90	0.04	2.40	0.08	2.47	0.07
				C	1.20		1.40		1.20		1.15		1.30	
A. melanogenuml	30	6	6.0	3T	1.77	0.04	2.20	0.06	2.37	0.08	2.90	0.10	3.23	0.12
				C	1.15		1.15		1.10		1.25		1.25	
A. oxydans 1	25	6	5.0	3T	2.10	0.06	9.43	0.53	14.80	0.85	32.97	1.95	41.27	2.45
				C	1.05		0.60		0.65		0.55		0.50	
A. oxydans 2	25	6	5.0	3T	2.40	0.08	24.93	1.45	44.67	2.64	46.20	2.75	44.43	2.64
				C	1.10		0.75		0.60		0.40		0.50	

APPENDIX TABLE XXVI

THE EFFECT OF VARYING THE SIZE OF THE INOCULUM IN YEAST EXTRACT MEDIUM ON THE AMOUNT OF ACETIC ACID PRODUCED

MAXIMUM YIELD PRODUCED AND TIME REQUIRED BY EACH ORGANISM FOR EVERY VARIATION IN SIZE OF INOCULUM

From Appendix Tables XXIII - XXV

Organism		Size of inoculum, standard		
		1	3 x^g	5 x
A. suboxydans	HAc/100 ml. medium, gm	0.05	0.08	0.05
	Time, days	11	10	8
A. acetosum	HAc/100 ml. medium, gm	2.45	2.48	2.56
	Time, days	6	8	6
A. ascendens	HAc/100 ml. medium, gm	2.45	1.97	2.08
	Time, days	11	10	10
A. pasteurianum	HAc/100 ml. medium, gm	0.14	0.11	0.11
	Time, days	12	10	10
A. turbidans	HAc/100 ml. medium, gm	0.04	0.06	0.07
	Time, days	12	6	8
A. kützingianum	HAc/100 ml. medium, gm	4.87	1.65	1.30
	Time, days	12	10	10
A. aceti 1	HAc/100 ml. medium, gm	2.66	2.47	2.73
	Time, days	8	10	10
A. aceti 2	HAc/100 ml. medium, gm	4.95	3.28	3.13
	Time, days	12	10	10
A. rancens	HAc/100 ml. medium, gm	4.28	1.28	1.54
	Time, days	11	10	10
A. gluconicum	HAc/100 ml. medium, gm	1.58	0.05	0.08
	Time, days	12	8	8
A. melanogenum 1	HAc/100 ml. medium, gm	3.59	0.11	0.12
	Time, days	10	10	10
A. oxydans 1	HAc/100 ml. medium, gm	4.77	2.42	2.45
	Time, days	11	10	10
A. oxydans 2	HAc/100 ml. medium, gm	4.77	2.90	2.75
	Time, days	11	10	8

g - x- times.

APPENDIX TABLE XXVII

THE EFFECT OF MIXED INOCULA IN THE YEAST EXTRACT MEDIUM ON THE AMOUNT OF ACETIC ACID PRODUCED

EACH ORGANISM + *ACETOBACTER ACETI* 1

Organism	Optimum Conditions				Readings									
					2 day		4 day		6 day		8 day		10 day	
	Temperature, °C	Ethyl alcohol, %	pH		Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm
A. suboxydans	35	3	6.0	3T C	1.63 1.00	0.04	2.07 0.75	0.08	2.73 0.70	0.12	3.30 0.65	0.16	3.80 0.55	0.20
A. acetosum	25	3	5.5	3T C	2.63 1.05	0.10	28.77 0.75	1.68	46.70 0.50	2.77	43.27 0.40	2.57	39.47 0.35	2.35
A. ascendens	25	3	6.0	3T C	1.87 0.90	0.06	11.57 0.60	0.66	36.53 0.35	2.17	40.07 0.25	2.39	41.97 0.45	2.49
A. pasteurianum	30	3	5.5	3T C	1.90 1.10	0.05	3.53 0.80	0.16	32.97 0.50	1.95	42.50 0.40	2.53	44.83 0.45	2.66
A. turbidans	35	9	5.5	3T C	1.60 1.05	0.03	1.73 0.85	0.05	1.70 0.80	0.05	1.67 0.70	0.06	1.70 0.60	0.07
A. kützingianum	25	6	5.5	3T C	1.73 0.90	0.05	2.07 0.55	0.09	2.67 0.50	0.13	8.83 0.40	0.51	25.50 0.40	1.51
A. aceti 1														
A. aceti 2	25	6	5.5	3T C	1.77 0.65	0.07	2.07 0.45	0.10	17.87 0.50	1.04	42.67 0.40	2.54	59.53 0.50	3.54
A. rancens	35	6	5.0	3T C	2.47 0.85	0.10	4.53 0.85	0.22	4.90[2] 0.80	0.25	7.33 0.70	0.40	19.83 0.70	1.15
A. gluconicum	25	3	5.5	3T C	2.00 1.00	0.06	16.07 0.85	0.91	29.27 0.45	1.73	33.60 0.40	1.99	52.47 0.40	3.12
A. melanogenum 1	30	6	6.0	3T C	1.60 0.85	0.05	1.87 0.75	0.07	2.27 0.40	0.11	2.63 0.30	0.14	2.90 0.40	0.15
A. oxydans 1	25	6	5.0	3T C	2.03 1.10	0.06	7.97 0.70	0.44	19.00 0.65	1.10	39.23 0.50	2.32	46.37 0.55	2.75
A. oxydans 2	25	6	5.0	3T C	2.43 1.15	0.08	27.07 0.55	1.59	58.80 0.50	3.50	59.97 0.55	3.57	54.87 0.55	3.26

APPENDIX TABLE XXVIII

THE EFFECT OF MIXED INOCULA IN YEAST EXTRACT MEDIUM ON THE AMOUNT OF ACETIC ACID PRODUCED

ACETOBACTER ACETI 1

Organism	Optimum conditions				Readings									
					2 day		4 day		6 day		8 day		10 day	
	Temperature, °C	Ethyl alcohol, %	pH		Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm
A. aceti 1	35	3	6.0	2T C	1.25 0.35	0.05[3]	2.08 0.30	0.11[5]			22.58 0.20	1.34		
	25	3	5.5	3T C	11.60 0.55	0.66	33.53 0.00	2.01	43.07 0.00	2.58	44.33 0.00	2.66	39.17 0.00	2.35[12]
	25	3	6.0	3T C	1.23 0.50	0.04	15.63 0.30	0.92	22.60 0.20	1.34	33.53 0.20	2.00	44.17 0.20	2.64[11]
	30	3	5.5	3T C	11.60 0.55	0.66	33.53 0.00	2.01	43.07 0.00	2.58	44.33 0.00	2.66	39.17 0.00	2.35[12]
	35	9	5.5	3T C	0.70 0.45	0.02	0.80 0.30	0.03	0.80 0.15	0.04	0.77 0.00	0.05	0.77 0.00	0.05
	25	6	5.5	3T C	0.97 0.65	0.02	1.13 0.25	0.05	1.40 0.00	0.08	1.70 0.00	0.10	1.90 0.00	0.11
	35	6	5.0	3T C	0.97 0.65	0.02	1.13 0.25	0.05	1.40 0.00	0.08	1.70 0.00	0.10	1.90 0.00	0.11
	30	6	6.0	3T C	0.97 0.65	0.02	1.13 0.25	0.05	1.40 0.00	0.08	1.70 0.00	0.10	1.90 0.00	0.11
	25	6	5.0	3T C	0.97 0.65	0.02	1.13 0.25	0.05	1.40 0.00	0.08	1.70 0.00	0.10	1.90 0.00	0.11

3 - Three day instead of two day reading.
5 - Five day instead of four day reading.

APPENDIX TABLE XXIX

THE EFFECT OF MIXED INOCULA IN THE YEAST EXTRACT MEDIUM ON THE AMOUNT OF ACETIC ACID PRODUCED

ALL ACETOBACTER ORGANISMS

Organism	Optimum conditions				Readings									
					2 day		4 day		6 day		8 day		11 day	
	Temperature, °C	Ethyl alcohol, %	pH		Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm
Thirteen together	25	6	5.5	3 T C	2.03 1.20	0.05	6.43 0.75	0.34	44.17 0.60	2.61	71.23 0.50	4.24	83.57 0.45	4.99

APPENDIX TABLE XXX

THE EFFECT OF MIXED INOCULA IN THE YEAST EXTRACT MEDIUM ON THE AMOUNT OF ACETIC ACID PRODUCED

MAXIMUM YIELD PRODUCED AND TIME REQUIRED BY EACH MIXTURE, FOR EVERY VARIATION IN ORGANISMS, AND COMPARISON WITH THE TEST USING INDIVIDUAL ORGANISMS

From Appendix Tables XXIX, XXVII, XXIII, XXVIII respectively

Organism		Mixture		Comparison, each organism	
		Organisms together	Organisms + *A. aceti* 1	Organism	*A. aceti* 1[h]
All *Acetobacter*	HAc/100 ml. medium, gm Time, days	4.99 11			
A. suboxydans	HAc/100 ml. medium, gm Time, days		0.20 10	0.05 11	1.34 8
A. acetosum	HAc/100 ml. medium, gm Time, days		2.77 6	2.45 6	2.66 8
A. ascendens	HAc/100 ml. medium, gm Time, days		2.49 10	2.45 11	2.64 11
A. pasteurianum	HAc/100 ml. medium, gm Time, days		2.66 10	0.14 12	2.66 8
A. turbidans	HAc/100 ml. medium, gm Time, days		0.07 10	0.04 12	0.05 8
A. kützingianum	HAc/100 ml. medium, gm Time, days		1.51 10	4.87 12	0.11 10
A. aceti 1	HAc/100 ml. medium, gm Time, days				
A. aceti 2	HAc/100 ml. medium, gm Time, days		3.54 10	4.95 12	0.11 10
A. rancens	HAc/100 ml. medium, gm Time, days		1.15 10	4.28 11	0.11 10
A. gluconicum	HAc/100 ml. medium, gm Time, days		3.12 10	1.58 12	2.66 8
A. melanogenum 1	HAc/100 ml. medium, gm Time, days		0.15 10	3.59 10	0.11 10
A. oxydans 1	HAc/100 ml. medium, gm Time, days		2.75 10	4.77 11	0.11 10
A. oxydans 2	HAc/100 ml. medium, gm Time, days		3.57 8	4.77 11	0.11 10

h - Maximum amount of acid produced under conditions of those used for the corresponding organisms studied.

APPENDIX TABLE XXXI

THE EFFECT OF VARYING THE CHEMICALS IN THE INOCULATED MEDIUM ON THE AMOUNT OF ACETIC ACID PRODUCED

MEDIUM 1 - INORGANIC SALTS + VITAMIN FREE CASAMINO ACIDS + SPECIAL AMINO ACIDS

Organism	Optimum conditions				Readings									
					2 day		4 day		6 day		8 day		10 day	
	Temperature, °C	Ethyl alcohol %	pH		Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm
A. suboxydans	35	3	6.0	3T C	1.48 1.30	0.01	1.77 1.15	0.04	1.95 1.25	0.04			2.28 1.30	0.06
A. acetosum	25	3	5.5	3T C	1.58 1.30	0.02	2.07 1.25	0.05	2.20 1.30	0.05			2.55 1.30	0.08
A. ascendens	25	3	6.0	3T C	1.48 1.35	0.01	1.78 1.20	0.04	1.83 1.30	0.03			2.32 1.20	0.07
A. pasteurianum	30	3	5.5	3T C	1.80 1.40	0.02	1.90 1.30	0.04	2.15 1.30	0.05			2.77 1.40	0.08
A. turbidans	35	9	5.5	3T C	1.45 1.30	0.01	1.63 1.50,	0.01	1.60 1.40	0.01			1.62 1.40	0.01
A. kützingianum	25	6	5.5	3T C			2.02 1.20	0.05	2.27 1.20	0.06	2.65 1.20	0.09	3.02 1.20	0.11
A. aceti 1	25	3	5.5	3T C	2.98 1.25	0.10	7.20 1.10	0.37	9.53 1.10	0.51			39.37 1.05	2.30
A. aceti 2	25	6	5.5	3T C	1.65 1.35	0.02	1.97 1.35	0.04	2.35 1.30	0.06			2.90 1.40	0.09
A. rancens	35	6	5.0	3T C	1.52 1.45	0.00	1.52 1.00	0.03	1.77 0.30	0.09			2.35 0.00	0.14
A. gluconicum	25	3	5.5	3T C	1.35 1.40	0.00	1.82 1.25	0.03	1.30 1.30	0.00			1.37 1.40	0.00
A. melanogenum 1	30	6	6.0	3T C	1.25 1.15	0.01	1.32 1.20	0.01	1.37 1.35	0.00			1.27 1.25	0.00
A. oxydans 1	25	6	5.0	3T C	1.82 1.45	0.02	2.20 1.40	0.05	2.62 1.35	0.08			13.97 1.00	0.78
A. oxydans 2	25	6	5.0	3T C	1.70 1.40	0.02	2.08 1.50	0.04	3.03 1.20	0.11			67.68 0.60	4.03

APPENDIX TABLE XXXII

THE EFFECT OF VARYING THE CHEMICALS IN THE INOCULATED MEDIUM ON THE AMOUNT OF ACETIC ACID PRODUCED

MEDIUM 11 - INORGANIC SALTS + VITAMIN FREE CASAMINO ACIDS + SPECIAL AMINO ACIDS + VITAMINS

Organism	Optimum conditions				Readings									
					2 day		4 day		6 day		8 day		10 day	
	Temperature, °C	Ethyl alcohol, %	pH		Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm
A. suboxydans	35	3	6.0	3T	1.43	0.01	1.75	0.03	1.98	0.04	1.90	0.03	2.03	0.04
				C	1.25		1.30		1.30		1.35		1.40	
A. acetosum	25	3	5.5	3T	1.40	0.01	1.75	0.03	2.10	0.05	2.30	0.06	2.77	0.09
				C	1.20		1.25		1.25		1.25		1.25	
A. ascendens	25	3	6.0	3T	2.00	0.06	2.25	0.08	2.37	0.08	2.37	0.08	2.93	0.11
				C	1.00		0.95		1.10		1.10		1.10	
A. pasteurianum	30	3	5.5	3T	2.15	0.06	2.53	0.08	2.68	0.09	2.97	0.10	3.48	0.13
				C	1.15		1.20		1.25		1.25		1.30	
A. turbidans	35	9	5.5	3T	1.48	0.01	1.37	0.00	1.60	0.00	1.65	0.00	1.75	0.01
				C	1.35		1.40		1.55		1.60		1.55	
A. kützingianum	25	6	5.5	3T	1.50	0.01	1.93	0.04	2.12	0.05	3.08	0.11	6.25	0.30
				C	1.30		1.30		1.30		1.30		1.30	
A. aceti 1	25	3	5.5	3T	3.90	0.17	5.77	0.29	7.98	0.42	11.95	0.65	16.23	0.91
				C	1.10		0.95		1.05		1.05		1.05	
A. aceti 2	25	6	5.5	3T	1.68	0.04	2.03	0.05	2.52	0.08	2.77	0.10	3.32	0.12
				C	1.10		1.15		1.15		1.15		1.35	
A. rancens	35	6	5.0	3T	1.53	0.00	1.50	0.00	1.62	0.00	1.77	0.00	1.78	0.02
				C	1.55		1.60		1.75		1.70		1.50	
A. gluconicum	25	3	5.5	3T	1.33	0.00	1.32	0.00	1.38	0.00	1.38	0.00	1.53	0.00
				C	1.30		1.40		1.45		1.40		1.60	
A. melanogenum 1	30	6	6.0	3T	1.20	0.01	1.15	0.00	1.33	0.00	1.32	0.00	1.42	0.00
				C	1.10		1.20		1.40		1.40		1.50	
A. oxydans 1	25	6	5.0	3T	2.23	0.05	3.03	0.11	4.38	0.19	7.38	0.38	16.37	0.92
				C	1.35		1.20		1.20		1.00		1.00	
A. oxydans 2	25	6	5.0	3T	1.67	0.02	2.47	0.06	4.17	0.17	7.00	0.34	33.58	1.93
				C	1.40		1.40		1.40		1.40		1.40	

APPENDIX TABLE XXXIII

THE EFFECT OF VARYING THE CHEMICALS IN THE INOCULATED MEDIUM ON THE AMOUNT OF ACETIC ACID PRODUCED

MEDIUM 111 - INORGANIC SALTS + VITAMIN FREE CASAMINO ACIDS + SPECIAL AMINO ACIDS + VITAMINS + PYRIMIDINES AND PURINES

Organism	Optimum conditions				Readings									
					2 day		4 day		6 day		8 day		10 day	
	Temperature, °C	Ethyl alcohol, %	pH		Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium, gm	Titration, ml	HAc/100 ml. medium,gm
A. suboxydans	35	3	6.0	3T	1.25	0.04	1.28[2]	0.04	1.37	0.05	1.53	0.06	1.38	0.05
				C	0.60		0.60		0.60		0.60		0.60	
A. acetosum	25	3	5.5	3T	1.00	0.03	1.25	0.04	1.32	0.05	1.98	0.09	2.43	0.11
				C	0.55		0.55		0.55		0.55		0.55	
A. ascendens	25	3	6.0	3T	1.10	0.03	1.40	0.05	1.60	0.06	2.00	0.08	1.92	0.08
				C	0.60		0.60		0.60		0.60		0.60	
A. pasteurianum	30	3	5.5	3T	1.23	0.04	1.43	0.05	1.63	0.07	1.97	0.09	2.13	0.10
				C	0.55		0.55		0.55		0.55		0.55	
A. turbidans	35	9	5.5	3T	0.75	0.01	0.73	0.01	0.70	0.01	0.60	0.00	0.75	0.01
				C	0.60		0.60		0.60		0.60		0.60	
A. kützingianum	25	6	5.5	3T	1.25	0.03	1.65	0.06	1.85	0.07	2.22	0.09	4.47	0.23
				C	0.70		0.70		0.70		0.70		0.70	
A. aceti 1	25	3	5.5	3T	2.27[2]	0.10	4.42	0.23	5.55	0.30	8.95	0.50	13.60	0.78
				C	0.55		0.55		0.55		0.55		0.55	
A. aceti 2	25	6	5.5	3T	1.12	0.03	1.38	0.04	1.63	0.06	2.20	0.09	3.18	0.15
				C	0.70		0.70		0.70		0.70		0.70	
A. rancens	35	6	5.0	3T	0.72	0.01	0.77	0.01	0.63	0.00	0.70	0.01	1.25	0.04
				C	0.58		0.58		0.58		0.58		0.58	
A. gluconicum	25	3	5.5	3T	0.70	0.01	0.60	0.00	0.55	0.00	0.62	0.00	0.67	0.01
				C	0.55		0.55		0.55		0.55		0.55	
A. melanogenum 1	30	6	6.0	3T	0.70	0.00	0.65	0.00	0.65	0.00	0.62	0.00	0.82	0.01
				C	0.63		0.63		0.63		0.63		0.63	
A. oxydans 1	25	6	5.0	3T	1.68	0.07	1.83	0.08	2.77	0.13	6.82	0.37	20.33	1.19
				C	0.58		0.58		0.58		0.58		0.58	
A. oxydans 2	25	6	5.0	3T	1.02	0.03	1.35	0.05	7.75	0.43	24.05	1.41	59.32	3.52
				C	0.58		0.58		0.58		0.58		0.58	

APPENDIX TABLE XXXIV

THE EFFECT OF VARYING THE CHEMICALS IN THE INOCULATED MEDIUM ON THE AMOUNT OF ACETIC ACID PRODUCED

MAXIMUM YIELD PRODUCED AND TIME REQUIRED BY EACH ORGANISM FOR EVERY VARIATION IN THE CHEMICALS OF THE MEDIUM

From Appendix Tables XXXI, XXXII, XXXIII and XXIII respectively

Organism		Medium			
		I [i]	II [j]	III [k]	IV [l]
A. suboxydans	HAc/100 ml. medium, gm Time, days	0.06 10	0.04 6	0.06 8	0.05 11
A. acetosum	HAc/100 ml. medium, gm Time, days	0.08 10	0.09 10	0.11 10	2.45 6
A. ascendens	HAc/100 ml. medium, gm Time, days	0.07 10	0.11 10	0.08 8	2.45 11
A. pasteurianum	HAc/100 ml. medium, gm Time, days	0.08 10	0.13 10	0.10 10	0.14 12
A. turbidans	HAc/100 ml. medium, gm Time, days	0.01 2	0.01 2	0.01 2	0.04 12
A. kützingianum	HAc/100 ml. medium, gm Time, days	0.11 10	0.30 10	0.23 10	4.87 12
A. aceti 1	HAc/100 ml. medium, gm Time, days	2.30 10	0.91 10	0.78 10	2.66 8
A. aceti 2	HAc/100 ml. medium, gm Time, days	0.09 10	0.12 10	0.15 10	4.95 12
A. rancens	HAc/100 ml. medium, gm Time, days	0.14 10	0.02 10	0.04 10	4.28 11
A. gluconicum	HAc/100 ml. medium, gm Time, days	0.03 4	0.00	0.01 2	1.58 12
A. melanogenum [l]	HAc/100 ml. medium, gm Time, days	0.01 2	0.01 2	0.01 10	3.59 10
A. oxydans 1	HAc/100 ml. medium, gm Time, days	0.78 10	0.92 10	1.19 10	4.77 11
A. oxydans 2	HAc/100 ml. medium, gm Time, days	4.03 10	1.93 10	3.52 10	4.77 11

i - See Appendix Table XXXI.
j - See Appendix Table XXXII.
k - See Appendix Table XXXIII.
l - See Appendix Table XXIII (ie. yeast extract medium).