2

23×9
207
16

S. Ranganayaki.

12-15 S. Ranganayaki.
2- Allahabad University

93
4
23-

R. No. 17285 of 1956
dated. 15/17 Dec. 1956.

Hilditch
chemistry of Natural fats

Ranganayaki.
Ranganayaki.
Ranganayaki.

nil/10

5.3 / 2.65 ... 0.55 × 5 / 2.65

5 c.c. → 50 c.c.

3rd Jan

	I SM 3% starch	II B.S. 6% starch
Sugar / Carbohyd	6.2 / 50 > not reduced	6.5 >
Protein hyd.	2.9 / 28.0 > 20.1	7.6 / 22.0 > 18.4
		0.5780
		A → 0.5780
		0.5776 → 0.5800
	0.7228	B → 0.5776
	0.7300 / 0072	
		13.7 / 15.0 > 1.3 c.c.
	alkali.	

SK Jaggery SM in 3% Sugar.

Sugar with hyd. 6.3 > no acid

Sugar hyd. 8 / 33.5 > 25.5

0.6400 >

S. marcescens.
Cane Sugar.

B. Subtilis.
Starch.

5th Dec. 1956.

Each Culture contained

Sugar — 3%
KH_2PO_4 — 0.15%.
K_2HPO_4 — 0.35%.
$(NH_4)_2SO_4$ — 0.17%.
(0.02% Nitrogen)
$MgSO_4$ — 0.08%.
Total volume — 100 c.c.

1 → 0.6800 .7228
2 → 0.7000 .6556
3 → 0.6350
4 → 0.6400

I
Son → Analysed on 3rd Jan.

On 6th Dec. 3% starch —
Analysed in 2nd Jan
6% Sugar
12% Sugar

3% Sugar — B.S.
6% } starch — B.S. II → 3rd Jan '57.
12% }

B → 0.5776
0.6200 .0424

1 → 0.5780
→ 0.6330 0.0550

ferm^n Oph temp. Relation
Butanol-acetone. 37 Anaerobic

30°.

Estimation:— S M in 6% Sugar S M in 12% Sugar.

5 → 50 c.c

Red sugar 6.2 } × 9.5 }
after hyd. 5.0
Total sugar 9.5

~~2-3 c.c~~ 2.5 } 5 c.c 8.0 } 3.8
12.5 11.8

~~Wt of~~ dinitro phenyl hydrazone. 0.6800 } 0.02 g. (3) 0.6556 } 0.01
0.700 0.6652

Acid in 5 c.c ~~0 c.c~~ 3.1 } 8.1 } 3.4 c.c
~~0 c.c~~ 6.7 11.5
11.6 }
13.6 } 2.0 c.c

NRRL B-527 Clostridium Acetobutylicum Mc. Coy et al.

B-598 " pasteurianum Bezssonoff

B-592 " butylicus Fitz.

B-510 RHG. Bacillus polymyxa (Prazmowski) Migula

B-905 Aeromonas hydrophila (Chester) Bergey et al

B-543 Bacillus subtilis Cohn emend. Prazmowski

B-284. Serratia marcescens. Bizio.

From.

United states department of agriculture
Agricultural Research Service
Northern Utilisation Research Brand
Peoria Illinois.

Carbohydrates 80 - 37. anaerobic Butanol-acetone

1. Bacillus subtilis.

2. S. marcescens (B. prodigiosum)

3. Clostridium butyricum.

4. Cl. acetobutyricum

5. Cl. butyricum 1352

6. Clostridium 174 (bildet Butter acid, acetic acid & butanol)

7. Cl. 208 (bildet butyric acid & acetic acid)

from

Biologische Bundesanstalt
für Land- und Forstwirtschaft
Berlin

Dr. H. Stolp

Analysis of cultures from 1 to 17

1-5 $ZnSO_4$
6-9 Alum
10-13 Antim
14-17 Carbon

Acid in c.c Titrated against exact N/20 Sod. carb.

(1) 0 / 0.5 > 0.5

(2) 0.5 / 1.2 > 0.7

(3) 1.2 / 1.6 > 0.4

(4) 1.6 / 1.8 > 0.2

(5) 1.8 / 2.1 > 0.3

(6) ~~2.1~~ / ~~2.6~~ 0.0 / 0.4 > 0.4

(7) 0.4 / 1.0 > 0.6

(8) 1.0 / 1.4 > 0.4

(9) 1.4 / 1.9 > 0.5

(10) 1.9 / 2.4 > 0.5

(11) ~~2.6~~ alk —

(12) 2.4 / 3.0 > 0.6

(13) 3.0 / 3.5 > 0.5

(14) 2.1 / 2.6 > 0.5

(15) 2.6 / 3.2 > 0.6

(16) 3.2 / 4.5 > 1.3

(17) 4.6 / 6.1 > 1.5

I to V.
XIII to (7)
Rest —

analysed on 7/4/58

8/4/58

Clostridium Butylicum. (p. 327 in P. Dunn).
gives good yield with waste sulphite liquors.

3 Prepare 3 cultures of B.S.

Reducing sugar. 25 → 50 c.c. Fehling 5+5 = 0.0545 g of sugar

(1) 11.5 / 16.5 → 5.0

(2) 11.4 / 18.0 → 6.6

(3) 11.7 / 16.9 → 5.2

(4) 14.5 / 19.9 → 5.4

(5) 11.7 / 19.1 → 7.4

(6) ~~0.1~~ 15.6 / 20.5 → 4.9

(7) 8.1 / 12.7 → 4.6

(8) 9.3 / 13.6 → 4.3

(9) 9.2 / 13.6 → 4.4

(10) 9.5 / 14.3 → 4.8

(11) 11.5 / 17.8 → 6.3

(12) 9.6 / 14.8 → 5.2

(13) 10.7 / 14.8 → 4.1

(14) 12.3 / 17.4 → 5.1

(15) 11.3 / 17.5 → 6.2

(16) 7.7 / 17.8 → 10.1

(17) 13.5 / 22.0 → 8.5.

Total Sugar in 5 c.c.

1) 41.1
2) 37.2
3) 31.8
4) 38.4
5) 29.5
6) 37.9
7) 39.7
8) 40.9
9) ~~31.8~~ 31.8.
10) 31.8 > 18.2 + 50 > 38.2
0 > 20.0
200
11) 43.2
12) 38
13) 37.7 – 37.7
14) ~~30~~ ← 34.6
15) 34.6 ← 33.8
~~16~~ 33.8
~~17~~
16) 41.7 – 41.7
17) 36.0 – 36.0

Bacillus poly myxa. (pH 5.6-6.5) (p 498)
30°C

Medium 1		Medium 2	
Soluble starch	2 %	Whole wheat	5 %
Yeast extract	0.5 %	Yeast extract	0.5-1.0 %
$CaCO_3$	1 %	$CaCO_3$	1 %

Yeast extract can be substituted by malt sprouts. dried yeast. ~~[illegible]~~

Butane diol as Nickel salt.

(1) 0.5848
0.6540
0.0692

(2) 0.6600
·7480
0.0880

(3) 0.6550
·7240
0.0690

(4) 0.6090
0.6690
0.0600

(5) 0.6574
0.7170 } 0.0596

(6) 0.6254
0.6846 0.0592

(7) 0.6408
0.6876 0.0468

(8) 0.6280
·6782

9- 0·6474 } nil
~~·7424~~ }

10- 0·6462 ~~—~~ ·7424

11 — 0·5914 nil

12— 0·6006.
·6472

13- ~~0·65~~ 0·6604
0·7420

14 — 0·6600
0·7090

15 — 0·6476 0·7046

16 — 0·6404 0·7070

17— 0·6450 0·7610

~~पीले~~ बात करो

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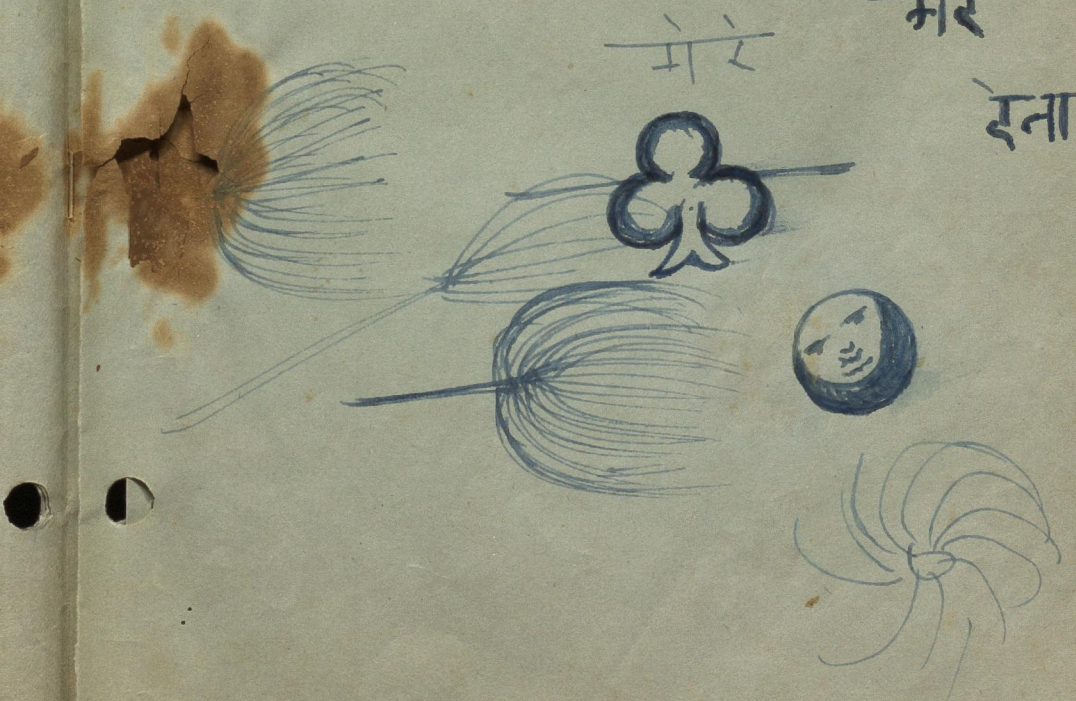


Cult medi for *B. Subtilis*. Date:- 12-12-56.

I

Starch — 24g. (6%) 400 c.c volume each.

KH_2PO_4 — 0.60 g

K_2HPO_4 — 1.40 g.

$MgSO_4$ — 0.32 %.

$(NH_4)_2SO_4$ — 0.68 g.

I

starch — 48 g.

III

starch — 96 g.

Seeded on.

These cultures are shift in 1lit Conical flasks. in 500 c.c.

Sugar cultures — 9.1.59.

I	II	III	
alk.	alk.	Oc.c 5.7 > 5.7.	Acid 2 c.c.
5 → 50			
6.8 >	7.7 >	7.7 >	Sugar Red.
5 hyd 3 min → 50 c.c			
9.4 > 15.8	9.5 > 11.4	8.4 > 22.6	Sugar Total
25.2	25.9	31.	
1 → 0.6500	2 → 0.6384	3 → 0.6646	
~~0.6350~~	0.6515	~~0.6750~~	
0.6840	0.6732	0.7100	
0.0240	0.0348	~~0.0454~~	
		(0.05)	
		(0.0459)	
7.0 >	5.1 >	5.7 > 31.8	Red Sugar [no dil]
		36.5	

Culture media for S. M.

1 Starch - 12 g.

KH_2PO_4 — 0.60 g

K_2HPO_4 — 1.40 g.

$MgSO_4$ — 0.32 g.

$(NH_4)_2SO_4$ — 0.68 g.

2. Sugar - 12 g.

2 → 0.05
5 → 0.1
10 → 2g

3 Cultures each of 100 c.c. milk (even number) 1, 2 & 4 c.c milk. (Sugar)

Starch (odd no.) 1, 2, 3 & 4 c.c milk

The six cultures Seeded on —

14. 12. '56.

Cultures. 11.1.57.

	II	III	IV	Acid
	alk.	26.7 / 28.2 → 1.5	alk.	
7.0 / 25.0 / 18.0	7.0 / 29.5	22.5 / 7.2 / 33.3 → 26.1	7.1 / 33.4 → 26.3	Total Sugar 5 → 50
4 c.c	5 c.c	16.0 / 36.1 → 20.1	10 / 18.8 → 6.8	Red Sugar Nil.
0.6600	0.6552	0.6342	0.6520	
0.6640	0.6620	0.6400	0.6620	
0.6800	0.6850	0.6550	0.6770	
0.0200	0.0302	0.0208	0.0250	

Mit bestem Dank bestätige ich
~~Ihren~~ den Empfang. Ihr

Six Cultures using $CaCO_3$ B.S. ~~B.S.M.~~ Lg. 12.56

Prepared 2 solutions A of KH_2PO_4 — 0.5 gm in 100 c.c.
B K_2HPO_4 — 0.5 g in 100 c.c.
Sterile $CaCO_3$ — ~~0.2~~ g. "

Each of Culture medium will contain
Starch 3% sucrose 3%

I	10 c.c A	10 c.c. B	~~10 c.c~~ C.
	10 c.c. A	20 c.c. B.	~~10 c.c.~~ C.
III	10 c.c. A.	20 c.c. B	~~10 c.c.~~ C.

Extraction :- ~~Extract~~ with K_2CO_3 saturated & extract with n- butanol or continuous extraction with di ethyl ether.

Analysis of B.S. Cultures. which were seeded on 8.1.57.

9.157

Starch

Analysed on 13.2.57.

10 c.c. Acid & alkali

1 2 3

Acid &

23.3 24.2 27.2

24.2 27.0 29.3 2.1

0.9 2.8 2.1

10 c.c. N/10 dichromate

0.1 10.3 22.3

10.3 10.2 22.1 11.8 29.9 7.6

(Bertrand's) Reducing Sugar 10 c.c.

1 0.1 11.6 11.5

2 11.7 10.4

3 22.2 30.5 8.3

Total Sugar

0.9 50. 0.3

Total sugar

16.4 41.5

41.9 30.6 48.9 18.3

33 5 8.6 9.9 9.3

16.5

I

0.6420

0.6460

0.6550

.0130

II

0.6320

0.6335

0.6460

.0140

III

0.6550

0.6500

0.6600

0050

Bacillus subtilis.

3% glucose, 1% yeast extract, 1% $CaCO_3$ - pH 6-6.8
30°C.

Thiamin [56.36% yld)
increases yield.

Lower pH is favourable
anaerobic conditions are favorable: N_2 gas conts no O_2.
1% glucose, 1.1 per cent peptone, 0.3% meat extract
1.5% disodium hydrogen phosphate (20.43% yld).

Serratia M.

1% peptone broth - 3% glucose buffered with excess sterile $CaCO_3$. [yld < 17%]

Anaerobic condns:

5% glucose, 0.5% yst extract, 0.05% KH_2PO_4. 0.05% K_2HPO_4.
0.02% $MgSO_4$ + 2% $CaCO_3$ separately sterilised [yld 50%]

16.2.57 Analysis of B.S. cultures [illegible] on 21.1.57

I	II	III	
2.5	3.0	alk	acid fm
9.6, 12.1 > 2.5	12.1, 15.1 > 3.0	alk red coloured bs.	
10.5	10.8	45 c.c	20 c.c → 50 c.c. red sugar
7.6, 18.1 > 10.5	7.4, 18.2 > 10.8	9.1, 50 +5 > 45 c.c	
	7.2 c.c	29.5 c.c	
8.4, 19.8 > 11.4; 23.3, 34.5 > 11.2	8.7, 38.2 > 29.5	8.1, 15.3 > 7.2	Total sugar

IV	V	VI	
0.6240, 0.7430, 0.7500 > 0.119	0.6280, 0.7480, 0.7530 > 0.12	0.6440, 0.7400, 0.7470	0.096 Phosph hyd

Weigh 3g starch in 6 flasks.

Prepare 100c.c of 85m contg. — 1.7g Amm sulphate
0.82g MgSO4

Weigh 12g Sugar
0.32 MgSO4
0.68 Amm SO4
50c.c of this +

.1774

Prepare 50c.c Phos. Make ~~700c.c~~ 35m

~~Starch~~

Starch Cultures of Serratia m. analysed on 16.1.57.

1	2	3	
5.750 1S	2S	3S	
~~6.3~~ 46.4	~~6.5~~ ~~47.4~~	6.6 60.	Sugar X hyd.
alk	alk.	14.3 16.0 1.7	Acid
15.3	16.6	17.7 48.5 30.8	Red Sugar no diln.
6.0 30.7 24.7	6.6 31.4 24.8	7.5 34.0 26.5	(Sugar after hyd. Red)

1 0.6490 0.6680 0.6700 0.0210	2 – 0.6934 2 – 0.7000 0.7050 ·7050 0.0116	3 – 0.6620 0.6600 0.6660 0.0040	Phenyl hyd

A Prepare ½ solutions. 1 of KH_2PO_4 - 100 c.c = [1.5 g / 3.5 g.]

·75
1.75

Six cultures of B.S & six of S.M to be prepared.
3 Sugar, 3 Starch [2 c.c milk] [3%]

Each will contain in 100 c.c. Starch Phos. soln

	B.S.	S.M.	
Sugar or starch — 3%	1B	1S	— 5 c.c.
$(NH_4)_2SO_4$ — 0.17 g.	2B	2S	— 10 c.c
$MgSO_4$ — 0.08 g.	3B	3S	— 15 c.c.

Starch cultures seeded on - ~~15 12.56~~
Sugar cultures seeded on -

B.S — 1B 2B 3B 1 2 3 (X) } All seeded on 18.12.56
S.M — 1S 2S 3S 1 2 3 (no mark)

[150 c.c flasks]

All the starch cultures of B.S got infected.
Again prepared on 8th Jan '57.
Sugar cultures of B.S on
Prepared 3 sugar cultures on 18.1.57
I II III seeded on 21.1.57

Contents [12 g Sugar / 0.32 $MgSO_4$ / 0.68 Am_2SO_4] 300 c.c. 50 c.c + (5, 10 & 15 c.c phos.) Tap water.

Sugar cultures of Serratia on 18.1.57.

I 4	II 5	III 6	
7.1, 19.8 > 12.7	6.6, 24.3 > 17.7	7.9, 45.6 > 37.7	Sugar aft. hyd
9.1, 12.9 > 3.8	4.7, 7.9 > 3.2	8.7, 22.6 > 13.9	Red sugar no diln.
17.6, 18.9 > 1.3	18.9, 25.2 > 6.3	~~12~~ alk.	Acid.

4 – 0.7000
0.7370
0.7440

5 – 0.6756
0.7650
0.0906
~~0.1~~
0.7750
0994

6 –
0.7172
0.7720
0.7800
7172
628

phenyl hydrazone

218

Prepare a solution of phosphates in 100 c.c.

KH_2PO_4 — 1.5 g

K_2HPO_4 — 3.5 g.

Add 5 c.c in each culture.

$CaCO_3$ — 2g.

Sugar or Starch — 3g.

$MgSO_4$ — 0.08g.

$(NH_4)_2SO_4$ — 0.1 g.

Make up volume — 100 c.c.

Prepare two cultures; one of B.S. one of S.M.

1 — ~~B~~ S.M. Sugar
2 — B.S. Sugar
3 — S.M. Starch
4 — B.S. Starch
2 c.c. milk added in SM.

Seeded on 22.12.56.

Analysed on 23.1.57.

	I	II	III	IV	
	7.1				Red. Sugar.
	~~7.10~~	7.6			20 → 50
	25.4 → 18.3	50 → 42.4	6.9 →	7.4 →	
	7.6	8.0			Total sugar
	17.3 →	28.4 →	14.0 →	6.9 →	20 → 50
I	0.6442	II 0.6342	III 0.6420	IV 0.7120	Phenyl hydryn
	0.6580	0.6584	0.6506	0.7100	
	~~0.6700~~			~~0.7200~~	
	0.0138	.0242	000 86		

The

Investigating ~~The~~ influence of concentration of carbon source in the 2.3. butanediol fermentation of Bacillus subtilis & Serratia marcescens

fermented liquid = F

(1) F (5 c.c) + d.n.p. ⟶ ppt.

(2) F " + HCl ⟶ boil. made alkaline and reduced with Fehling's soln. Boil for 2 minutes Acidify with d HCl. Add d.n.p ⟶ ppt is obtained.

(3) F + conc HCl 2 drops. Boil for a minute Add Fehling's soln. After complete reduction of Feh. Soln. Filter. Acidify filtrate with HCl add d.n.p ⟶ ppt is obtd. dark brown in colour, [much less in quantity than 2]

(4) F + Fehling soln. Boil & filter & add d.n.p. Very little ppt, blackish, less in quantity than (3) is obtd.

(5) d.n.p + excess Fehling's soln. Boil. After very long time a small quantity of ppt is obtained.

(6) To the solution 5 c.c F is heated with 5 c.c Br_2 & 5 c.c $FeCl_3$ in a sealed tube and then heated with d n.p beautiful yellow colour ppt is obtd.

1. Dextrin — By estimating with Fehling solution.

2. Reducing sugars — By estimating the sugars after hydrolysing with Hcl.

To 10. c.c. of the culture add 5 c.c. of Hcl and boil the mixture for 2 mins. Cool, make it alkaline, make the total vol. to 100 c.c. and estimate the sugar.

3. Acid — Only in those cultures which do not contain $CaCO_3$.

4. 2, 3, butyric acid.

$MgSO_4$ — 0.32 g.
$(NH_4)_2SO_4$ — 0.68 g.
Starch — 12 g.

Prepare $ZnSO_4$ 80 in 1 gm 100 c.c.

1, 2 & 5 c.c
~~5, 10, 15~~
Sugar 12 g.

Add 50 c.c. of Mix
5 c.c of Phos.
45 c.c. water.
5 c.c
$CaCO_3$ — 2 g.
$ZnSO_4$ —

7th Jan

Effect of $ZnSO_4$.

Prepare a soln of phosphates in 100 c.c

for 5 months
of May.

KH_2PO_4 — 1.5g
K_2HPO_4 — 3.5g

Add 5 c.c in each culture.

$CaCO_3$ — 1g

Sugar or Starch — 3g.

$MgSO_4$ — 0.08g

$(NH_4)_2SO_4$ — 0.17g.

(0.5g in 50 c.c.) $ZnSO_4$ — 1, 2 & 3 c.c

$1\,c.c = \frac{0.5}{50} = \frac{1}{10} = .01$

Six cultures for Bacillus Subtilis. (3 of Sugar 3 of starch)

4 5 6
I II III Starch Cultures

(1) (2) (3) Sugar cultures.

(250 c.c flasks) Seeded on 8.1.57.

Analysed on — 7.2.57

	1.		2		3		4		5		6
titr. with Ncl 5 c.c 80% after shaking for CaCO3 content.	0.5	0.2	29.2	1.2	0.7	0.2	5.2	1.1	6.3	0.4	6.7	0.3
	0.7		30.4		0.9		6.3		6.7		7.0	
titr. with alk.												
Red sugar 20 c.c → 50 c.c	8.0	17.0	6.7	15.7	6.8	43.2	7.0		6.5		6.8	
	25		22.4		50							
total sugar 20 c.c → 50 c.c	7.5	15.6	6.9	14.5	8.7	23.0	6.9		7.3		8.5	
	8.1		7.6		4.3							
phenyl hydrazone	0.6547	0.7262	0.6190	0.709	0.6242	0.6850	0.6300	0.6350	0.6070	0.6126	0.6450	0.001
		0.0715		0.0900		0.0558		0.0050		0.0056		

8 Authors

10/1/57 8 Soreon cultures each conty.

Sugar — 3g × 10 = 30 gm. [500 c.c.]

KH_2PO_4 } 1.5 | 2 → 0.75
K_2HPO_4 } — 3.75 | 2 → 1.875

$MgSO_4$ — 0.8 g

$(NH_4)_2SO_4$ — 1.7 g

~~$CaCO_3$ — 1 gm each flask.~~

Take 50 c.c in 8 flasks. Make up 100 c.c with water Seed with 1 → 8

Prepare 12 Cultures: [650 c.c] Weigh → 39 Sugar

KH_2PO_4	.075 × 13 →	0.975 g
K_2HPO_4	1875 × 13 →	2.4375 g
$MgSO_4$	0.08 × 13 →	~~0.104 g~~ 1.04 g
$(NH_4)_2SO_4$	0.17 × 13 →	2.21 g

.4375
8.
.4455

Seeding — 16.1.57.

Analysed — (1 ⊖ 6) → 1.2.57
(7 ⊖ 12) → 2.2.57

	I	II	III	IV	V	VI
Acid 10 c.c	alk	0 → 2.2	alk	alk	2.5 → 2.8 (5.3)	5.3 → 2.6 (7.9)
Red Sugar 20→50	7.9 →	X twice 8.1 → 50 + 50	2.9 →	X twice 8.2 → 50 + 50	8.0 → 14.5	7 → twice
Total Sugar 20→50	8.5 → 4.9 (13.4)	7.5 → 4.2 (11.7)	7.3 → 5.2 (12.5?)	7.5 → 5.0 (12.5)	7.7 → 6.1 (13.8)	6.8 → 5.0 (11.8)
Pheph high	0.6 / 0.64 — 0.04	0.64 / 0.6680 — 0.0280	0.6250 / 0.6650 — 0.0400	0.6030 / 0.6280 — 0.0250	0.6300 / 0.7000 — 0.0700	0.6520 / 0.6600 — 0.0100

	7	8	9	10	11	12
Acid	0.8 →					

	7	8	9	10	11	12
Acid loc. c	0.8 4 > 3.2	4.3 5.8 > 1.5	5.8 7.8 > 2.0	33.8 35.4 > 2.4	alk	alk.
Red Sugar 20→50	8.5 32.7 > 24.2	X 8.7 >	8.2 15.2 > 7.0	7.4 14 > 6.6	7.5 >	8.2 >
Total Sugar 20→50	8.6 13.6 > 5.0	8.0 13.5 > 5.5	~~7.8 > 6.2~~ 14.0 14.7 20.9 > 6.2	8.8 14.8 > 6.0	8.2 12.6 > 4.4	6.9 11.5 > 4.5
Phenyl hydr.	0.6150 0.6700 0.0550	0.6250 0.6720 0.0470	0.6460 0.7180 0.0720	0.6340 0.7070 0.0730	0.6070 0.6342 0.0272	0.6186 0.6554 0.0372

17.1.59 8 Cultures for Bacillus Subtilis.

Each containing — g

KH_2PO_4 – 0.15

K_2HPO_4 – 0.35

$MgSO_4$ – 0.088

$Am\ SO_4^{--}$ – 0.17g.

Prepare 500c.c. of Phos. soln. contg

KH_2PO_4 — 1.5 g $MgSO_4$ – 0.8 gm

K_2HPO_4 – 3.5 g $(NH_4)_2SO_4$ – 1.7 gm.

Add 50 c.c in each Culture.

	(1)	(2)	(3)	(4)
Add in 4 Sugar —	3g.	6gm.	9gm.	12gm.
4 starch —	3g.	6gm.	9gm.	12gm.
	I	II	III	IV

Prepare 100c.c. cultures.

The cultures were seeded on — 25.1.59.

Analysed on 1 → 4 — 6.3.57

I → IV — 8.3.57

I 29.8820 II 24.668

29.9300 24.68

.0480 .028

.048 × 20 × 0.824 =

0.028 × 20 × 0.824 =

~~महावीर से जैसा कपड़ा हो मंगवाना है~~

1	2	3	4	
12.8 → 14.6 → 1.8	15.0 → 18.0 → 3.0	18.2 → 20.8 → 2.6	20.8 → 23.3 → 2.5	Acid in 5 c.c
0.6 → 13.8 → 13.2	13.8 → 39.9 → 26.1	2.8 → 30.8 → 28.0	1.7 → 37.4 → 35.7	Red dye in 5 c.c
(4) 12 gm. 12 gm. IV	0.2 → 13.6 → 13.6	13.6 → 27.1 → 27.2 → 13.5	27.2 → 50 → 22.8; 8.8 + 50 → 41.2; 6.3 + 0.3 → 6.6 → 70.3	6.7 → 50 → 43.3; 0.1 → 49.9; 27.2 → 50 → 3.7 → 46.9	Total dye in 5 c.c
				0 → 50 + 0.4 → 7.1	Phenol hydrg

Bromine completely reacted decolourised in 3 & 4

1	2	3	4
0.704	0.6570	0.7040	0.6860
0.800	0.7500	0.7980	0.7680
0.096	0.0930	0.0940	0.0820

1S 2S 3S. marked (3)
Seeded on 21.1.59
Contains diff concns of Phos asbefore
5, 10 & 15 c.c 80% in order
Sugar – 2%
Seeded with — Aerobacter aerogenes
M-148 (Oxford)

Prepared like this
50 c.c Phos. soln conts
KH_2PO_4 — 0.75
K_2HPO_4 1.75

1S – 5 c.c, 2S – 10 c.c. 3S – 15 c.c

Sugar – 12g
$MgSO_4$ – 0.52
$(NH_4)_2SO_4$ – 0.68
dissolved in 300 c.c water
50 c.c added to each.

Made up to 100 c.c with water

X 21.1.57. was seeded with 10.
one culture — Sugar 3.1. of

KH_2PO_4 - 0.15

K_2HPO_4 - 0.35

$(NH_4)_2SO_4$ - 0.17.

$MgSO_4$ — 0.08.

Starch Cultures.

	I	II	III	IV	
Acid in 5c.c	25.3 26.8 > 1.5	26.8 28.5 > 1.7	28.5 30.4 > 1.9	30.4 31.8 > 1.4	
Red Sugar in 5c.c	0c.c. 4.4c.c > 4.4	4.4 15.7 > 11.3	15.7 25.3 > 9.6	25.3 29.4 > 4.1	
Total Sugar in 5c.c	? 29.40 35.5 > 6.1	1.0 21.4 > 20.4	21.4 > 50 + 0.4 1.9 > 28.6 1.5 30.1	1.9 23. > 21.1	

⑤ 0.6420 ⑥ 0.6820 ⑦ 0.6920 ⑧ 0.6760

0.6750 0.69

Phenyl hydra.

25th. Prepare 6 cultures for Serratia marcescens

Repetition

I Prepare phosphate solution 100 c.c.

KH_2PO_4 — 1.5g.

K_2HPO_4 — 3.5g. Solution A.

Prepare 350 c.c. solution containing

Sugar — 3×7 = 21g.

$(NH_4)_2SO_4$ — 0.17×7 = 1.19g

$MgSO_4$ — 0.08×7 = 0.56g. → [350 c.c soln added.]

Take 50 c.c of ~~each~~ this in six flasks.

In 1, 2 & 3 add 5, 10 & 15 c.c of A and make up to 100 c.c.

In ~~I, II & III~~ add 5, 10 & 15 c.c. of A and 2 c.c of milk and make up to 100 c.c

Sterilise and seed with SM. (8)

IV Cultures seeded on — 31.1.57

A culture marked nil cont

50 c.c soln

10 c.c phos.

4 c.c. milk.

Seeded with S.M on — 31.1.57

flat bottom flasks

I II III IV

27.3.57

alkaline alkaline

0.3 → 4.6 → 4.3

4.6 → 8.3 → 3.7

8.3 → 12.9 → 4.6

12.9 → 19.6 → 6.7 in 10 c.c Red Sugar

2.3 → 48.2 → 47.9

0.4 → 49.9 → 49.5 + 1.4 = 8.2 → 14.8

14.8 → 50 → + 35.2

0.5 → 50 → + 49.5

0.6 → 6.4 → 6.4

6.5 → 50 → +

0.4 → 8.4 → 43.4 → 8.0

Total Sugar in 10 c.c.

47.9 64.1 91.1 51.4

① 0.6470
0.6630
0.0160
283.59
1

② 0.6750
0.6900
0.0150

③ 0.6830
0.7000
0.0170

⑦ Phenylhyd.
0.6450
0.6570
.0120

2 3 all alkaline

0.2 → 2.2 → 2.0

2.2 → 3.6 → 1.4

3.6 → 6.3 → 2.7 Red Sugar in 10 c.c

4

6.3 → 50 → + 43.7

0.5 → 1.6 → 1.1

44.8

5

1.8 → 50 → + 48.2

0.3 → 2.3 → 2

50.2

6

2.5 → 48.5

46.0

Total Sugar in 5 c.c.

Prepare 12 cultures. Comp. Study.

Prepare 650 c.c contg.

Starch — 3g × 13 → 39 gm.

KH_2PO_4 — 0.15 × 13 → 1.95g.

K_2HPO_4 — 0.35 × 13 → 4.55g.

$(NH_4)_2SO_4$ — 0.17 × 13 → 2.21g.

$MgSO_4$ — 0.08 × 13 → 1.04g (Made upto 650)

Take 50 c.c in 12 flasks add 50 c.c water, cotton plug & Sterilise.

Seed from 1 → 12.

Seeded on — 1.2.57.

Analysed on — 1 → 6 → 28th Feb. 57

7 → 12 — 1st March 57.

A	B	C	
(4)	(5)	(6)	Phenyl hydrazone
0.6850	0.6580	0.6780	
0.7050	0.6700	0.7024	
.0200	.0120	0.0244	

1 2 3 4 5 6

acid (10 c.c aq. NH_2CO_3)

9.7
14.6 > 4.9

~~25.5~~
~~23.1~~
40.2
48.9 > 8.7

29.7
33.8 > 4.1

alk

~~27.8~~ 33.8
40.2 > 6.4

alk

0.1
8.5 > 8.4

8.5
15.5 > 7.0

16.0
24.1 > 8.1

24.1
50 > 0.3 + 1.9 > 27.5
25.9
1.6

1.9
14.1 > 12.2

14.3
21.3 > 7.0

red. Sugar in 10 c.c

0.2
18.3 > 18.1

18.3
35.1 > 16.8

0.5
20.6 > 20.1

20.6 > 08 + 14.4
50

14.5
35.5 >

6.6
35.8 >
29.2

Total Sugar in 10 c.c.

29.4 +
13.6 → 43.0 21.0

phenyl hydra.

	I	II	III	IV	V	VI
	0.6300	0.6340	0.6680	0.6800	0.6360	0.6680
				0.7640	0.7140	0.6360
	0.001	0.001	0.001	0.0840	0.0780	0.0360

Mard 1913

7	8	9	10	11	12	
1.3 / 6.1 > 4.8	6.1 / 7.2 > 1.1	9.4 / [illegible] 35.7 / 46.9 > 11.2	23.2 / 27.7 > 4.5	alk	29.8 / 35.7 > 5.9	~~10 c.c~~ acid in 10 c.c.
0.3 / 11.1 > 10.8	12.1 / 32.2 > 20.1	2.2 / 28.2 > 26.0	28.3 / 37.0 > 8.7	37.1 / 40.2 > 3.1	40.3 / 42.7 > 2.4	Red Sugar in 10 c.c
0.2 / 19.3 > 19.1	19.3 / 47.5 > 28.2	0.4 / 28.8 > 28.4	1.2 [illegible] / 20.0 > 18.8	20.0 / 30.2 > 10.2	30.2 / 39.8 > 9.6	Total sugar in 10 c.c
0.6900	0.6380	0.6560	0.6730	0.6170	0.6460	

Room temp

Prepare 6 cultures for SM. All containing 1gm of $CaCO_3$. ~~4 80/0 sugar~~.

Prepare 100 c.c. phos. solution

1.5 g — KH_2PO_4

3.5 g — K_2HPO_4

Put 1 4 — 5 c.c

2 5 — 10 c.c

3 6 — 15 c.c.

To 1, 2 & 3, add 2 c.c milk.

To each add 50 c.c of soln. from A

Dissolve in 350 c.c

2 1g — Sugar

1.19 g $(NH_4)_2SO_4$

0.56 g — $MgSO_4$.

Make up each to 100 c.c.

Cultures prepared on — 13.2.57

~~Prepare 2 cultures without $CaCO_3$.~~

~~Add to one milk~~

Analysed on — 15.4.57

II

I	II	III	IV	V	VI	
0·3 / 11·7 → 11·4	11·7 / 43· → 31·3	0·1 / 8·1 → 8·0	8·1 / 12·2 → 4·1	12·2 / 18·6 → 6·4	18·6 / 21·8 → 3·2	Calcos left with Lich Cultur 10 c.c.
0 / 22·1 → 22·1	22·1 / 33·3 → 11·2	33·3 / 40·0 → 13·7	0·3 / 17·9 → 17·6	17·9 / 21·3 → 3·4	21·3 / 37·9 → 16·6	Red boyin 5c.c
0·0 / 25·6 → 25·6	25·6 / 46·9 → 21·3	0·0 / 16·3 → 16·3	16·5 / 42·9 → 26·4	0·0 / 7·4 → 7·4	7·4 / 22·9 → 15·5	Total boyin 5c.c

I	II	III	IV	V	VI
0·6800	0·6770	0·6560	0·6620	0·6780	0·6760
0·7740	0·7700	0·7550	0·7350	0·7220	0·7670
0·1060	0·0930	0·0990	0·0730	0·0440	0·0910

Room temp.

Prepare 12 cultures with 3% Sugar & ~~milk~~,
10 c.c. phosphate soln. -

Sugar — 39 gms.

KH_2PO_4 — 1.95 g. + 31.3186 = 33.2686

K_2HPO_4 — 4.55g 4.55 = 35.8686

$(NH_4)_2SO_4$ — 2.21g. = 33.5286

$MgSO_4$ — 1.04 g Make upto 650 c.c
32.3586

Take 50 c.c in 12 flasks. add 50 c.c water.
cotton plug & sterilise.

Seed from 1 → 12.

Cultures seeded on —— . 23.2.57.

discarded

June no/: 6 got infected (SM from Ottawa)

No/: 1 Slightly infected Analysed.

Cultures 1 to 5 & 7 analysed on
—— 4.4.57

III

8 to 12 analysed on
——

I. II III IV V VI Calc. left

1 2 3 4 5 7

3.5 / 6.7 → 3.2 | 6.7 / 10.9 → 4.2 | 10.9 / 13 → 2.1 | alk | 13.0 / 15.7 → 2.7 | 15.7 / 19.6 → 3.9 | Acid in 10 c.c

1.3 / 16.8 → 15.5 | 16.8 / 24.9 → 8.1 | 25.0 / 32. → 7.0 | 34.8 / 38.2 → 3.4 | 0.8 / 44.0 → 43.2 | 3.0 / 35.9 → 32.9 | Red'n in 10 c.c

8.3 / 50 → 41.7 | 1.0 / 42.8 → 41.8 | 1.1 / 50 → 48.9 | 2.0 / 44.0 → 42.0 | 0.4 / 50 → 49.6 | 6.6 / 50 → 43.4 | Total red'n in 10 c.c

+ 6.9 / 22.2 → 15.6 / 57.3 | + 0.1 / 2.0 → 1.9 / 50.8 | + 0.2 / 6.5 → 6.3 / 55.9 | 1.2 / 8.3 → 7.1 / 51.5

I	II	III	IV	V	VI	
0.6520	0.6580	0.6440	0.6620	0.6550	0.6070	Phenyl hydrazone
0.7170	0.6760	0.7040	0.6850	0.7800	0.7140	
.0650	.0180	.0600	.0230	.0250	0.1070	

8	9	10	11	12	Acid in 10 c.c.
9.0 → 11.7 → 2.7	alk.	11.7 → 16.3 → 4.6	16.3 → 19.4 → 3.1	19.4 → 21.6 → 2.2	
~~0 c.c → 25.5 c.c~~	29.8 → 33.9	0 c.c → 27.5	33.9 → 38.4	38.4 → 41.6	Red Sugar in 5 c.c
28.9 → 29.8 → 0.9	4.1	27.5	4.5	3.2	
0.6 → 40.8 → 40.2	0.4 → 50 → 49.6; 0.3 → 35 → 34.7; 84.3	0.3 → 46.4 → 46.1	0.9 → 50 → 49.1; +0.8 → 36.5 → 35.7; 84.8	0.4 → 50 → 49.6; 29.8 → 37 → 7.2; 56.8	Total Sugar in 5 c.c.
40.2	84.3	46.1	84.8	56.8	

⑧	⑨	⑩	⑪	⑫
0.6650	0.6220	0.6840	0.6600	0.6130
0.7270	0.6270	0.7760	0.6630	0.6460
.0630	0.0050	.0920	.0030	.0330

Prepare six cultures of Serratia marcescens

Weigh — Rough

Repetition

Sugar — 21g ~~Sugar~~

$(NH_4)_2SO_4$ — 1.19 g

$MgSO_4$ — 0.56 g

KH_2PO_4 — 0.15 x 7 = 1.05 g

K_2HPO_4 — 0.35 x 7 = 2.45 g

Make up to 350 c.c.

Take 50 c.c ~~of~~ in six flasks.

Add milk 1 — nil

2 — 1

3 — 2

4 — 3

5 — 4

6 — 5.

IV

Cultures seeded on —— 8-3-57

Analysed on —— 17.4.57

I	II	III	IV	V	VI
1.6 / 3.7 → 2.1	3.7 / 6.9 → 3.2	6.9 / 9.6 → 2.7	9.6 / 12.5 → 2.9	12.5 / 15.5 → 3.0	15.5 / 19.2 → 3.7
0.0 / 3.9 → 3.9	3.9 / 10.9 → 7.0	10.9 / 24.0 → 13.1	24.0 / 40.6 → 16.6	40.6 / 50 → 9.4; 0 / 8.1 → 8.1; 9.4 + 8.1 = 17.5	8.3 / 18.8 → 5.5
0.0 / 50 → 50; 0.0 / 6.4 → 6.4; 50 + 6.4 = 56.4	7.4 / 50 → 42.6; 37.6 / 50 → 12.2; 0.1 / 18.3 → 18.2; 42.6 + 12.2 + 18.2 = 73.0	18.3 / 50 → 31.7; 0.0 / 12.0 → 12.0; 31.7 − 12.0 = 43.7	12.0 / 50 → 38.; 0.3 / 8.1 → 7.8; 38. − 7.8 = 45.8	8.1 / 48.6 → 40.5	0.0 / 48.1 → .
56.4	73.0	43.7	45.8	40.5	48.1
0.7830 − 0.7450 = .0420	0.6920 − .7480 = .0560	0.6725 − 0.7470 = 0.0745	0.6660 − 0.7530 = 0.0930	0.6774 − 0.7700 = .0926	0.6740 − 0.7430 = .0690

Prepare 12 solutions with 3.1 sugar & 2 c.c milk.

Acid in 5c.c

Red Sugar in 5c.c

Total Sugar in 5cc.

Prepare 12 cultures. with 3% Sugar + 2 c.c milk

Sugar — 39 g.

Room temp

KH_2PO_4 — 1.95

K_2HPO_4 — 4.55

$(NH_4)_2SO_4$ — 2.21

~~$MgSO_4$ — 1.04~~ Make up to 650 c.c

Take 50 c.c in each flask.

Dissolve 1.6 ~~0.5~~ $MgSO_4$ in 100 c.c Add 5 c.c in each flask Add 43 c.c water. Total 100 c.c.,

Add 2 c.c milk in each flask

Cultures seeded on — 2-4-52.

Analysed on —— 26.4.52.

Mark of Tube V

1	2	3	4	5	6	
3·7 6·0 ⟩ 2·3	6·0 17·6 ⟩ 11·6	17·6 23·1 ⟩ 5·5	23·1 24·7 ⟩ 1·6	24·7 30·6 ⟩ 5·9	30·6 34·6 ⟩ 4·0	Acid in 10 c.c.
0·6 4·7 ⟩ 4·1	4·7 12·9 ⟩ 8·2	12·9 19·0 ⟩ 6·1	19·0 23·9 ⟩ 4·9	23·9 32·3 ⟩ 8·4	32·3 37·0 ⟩ 4·7	Red Sugar in 5 c.c.
0·0 4·3 ⟩ 4·3 50	4·3 50 ⟩ 45·7 0·1 ⟩ 49·9 50 ⟩ 2·2 0·0 ⟩ 97·8 2·2	2·2 8·8 ⟩ 6·6	8·8 28·8 29·0 29·0 43·4 ⟩ 14·4	28·8 43·4 50 ⟩ 6·6 12·8 33·7 46·5 ⟩ 19·4	8·8 29·0 ⟩ 20·2	Total Sugar in 5 c.c.
0·6780 0·7010 0·0230	0·6444 0·6582 ·0138	0·6756 0·7136 ·0380	0·6502 0·6644 ·0142	·6444 ·620 ·026	·6540 0·6630 ·0090	

I. II III IV. V. VI Calc Alb

7 8 9 10 11 12

Acid 10 c.c

3.1 1.5 19.6 10.5 15.9 18.1
19.6 7.7 6.2 50 15.8 18.1 27.6
27.6 22.4 5.4 2.2 9.5
50

0.6 30. 30.6 10.1 40.7 6.2 0.0 7.3 7.3 4.9 12.2 3.8
30.6 40.7 46.9 7.3 12.2 16.0

Sugar 10 c.c

16.0 34 14.6 35.4 19.4 32.8 18.5 31.5 12.6
50 14.6 50 19.4 50 12.1 21.0
0 0 0.5
14.6 48.6 19.4 54.8 13.4 18.8 23.9 12.6 43.6 8.4

Total Sugar 50 c c

.6460 .6364 .6650 0.6456 0.6514 0.6282
0.6854 0.6456 .6730 0.6750 0.6740 0.6470
.0384 .0092 .0080 .0294 .0226 .0188

Prep are ~~8~~ 8 cultures of B.S. cntg $ZnSO_4$.

Prepare a soln of $ZnSO_4$. 1g in 100 c.c.

Add 5 c.c Phos.

Culture no 1 : 1 → no $ZnSO_4$ ← no 5 Add 10 c.c Phos

2 → 1 c.c ← no 6

3 → 2 c.c ← 7

4 → 3 c.c. ← 8

Add

Prepare ~~six~~ 12 cultures of SM.

5, 10 & 15 c.c phos.

3 milk 2 c.c milk

3 milk ~~out~~ milk

3 milk 2 c.c milk + 1gm $CaCO_3$

3 milk ~~out~~ milk + 1gm $CaCO_3$.

Analyse after 15.2 days.

Weigh following:—

Prepare 2 sets. ~~in one Carbon source is 10%~~.
Of each cmty optimum quantity of sugar
phos. & milk. (Opt. the considered milk substitutes
Standard X

I Make 6 ~~set~~ cultures with 10% sugar and
analyse them at the interval of 15 days each

II Make 6 cultures cmty 5% sugar and
analyse at 15 days each.

Concentration of phosphate should be optimum
quantity of milk optimum. Cultures should be
kept at room temp and ~~temp~~ recorded.

Organism:— best three. found so far.

X In one set market sugar
In other molasses sugar used as the
source of carbon.

All the organisms are to be seeded
separately (in chuna Dhan yeast).
25 cultures in each set.

Estimate the following in 24 hours. Take 50 c.c. ~~100 c.c.~~ 50 c.c.

Estimate sugar. (Red & Total)
Acid

116

Six cultures should be prepared cntg Opt. quantity of Carbohydrate, phosphate & milk & to be seeded by 1 c.c, 10 c.c, 20 c.c and 40 c.c and 80 c.c of the seed culture. The total volume should be 200 c.c. The seed culture should be prepd separately in a flask and shld be incubated for 1 week before seeding. The seed culture shld not contain more than 1% of sugar to begin with. Before seeding with this culture, estimate quantities of alcohol & sugar and evaluate the quantity of alc & sugar introduced in each culture & account for this during final calculation

Weigh the following for sugar cultures

A) Prepare a solution of phosphates

KH_2PO_4 – 0.15 × 30 = 4.5 g

K_2HPO_4 – 0.35 × 30 = 10.5 g

in 300 c.c.

B) Prepare a solution of Amm. sulphate & $MgSO_4$

$MgSO_4$ – 0.08 × 30 = 2.4 g

$(NH_4)_2SO_4$ – 0.17 × 30 = 5.1 g

in 300 c.c

C) Prepare a solution of sugar

Dissolve in 300 c.c

30 × 5 = 150 gm.

~~Milk~~ 3 cc per culture.

A – 10 c.c

B – 10 c.c

C – 10 c.c.

100
33
67

Add — 270 c.c. water & make up to 100 c.c

Seeded on 3rd May 1957.

Hg phenyl hydrazine

1	2	3	4	5	6
0.6580	0.6835	0.6640	0.6560	0.7030	0.6700
0.7030	0.7430	0.72	0.7280	0.7820	0.7480
.0450	.0595	.0560	0.0720	0.0790	.0780
	.0450	.0450	.0450	0.0450	.0450
	0.0145	6110	.0270	0340	.0330

26 Cultures were made using ten ~~four~~ times diluted molasses ~~[illegible]~~. They were seeded with the different organisms.

The cultures showed no growth of the organisms. Seeded on 3rd May

Analysed on – 16th July.

5 c.c of soln for acid, Phenylhyd. Red sugar + Total sugar

Nos I ~~[illegible]~~ X 24, 26, 19 & 22 got infected (bad bad bad bad bad. (bacteria))

Control	II	III	IV	V	VI	
0, 18.4 > 18.4	18.8, 30.5 > 11.7	30.5, 36.0 > 5.5	0, 6 > 6.0	7.8, 23.0 > 15.2	23.0, 42.3 > 19.3	Acid N/50 Na_2CO_3
0, 50 >; 0, 28 > 78	0, 50 > 50; 34.8, 44.1 > 9.3, 59.3	0.2, 50 > 41.8; 14.8, 21.3 > 6.5	21.3, 50 > 28.7; 0.0, 7.1 > 48.3	7.1, 7.1 > 39.8; 35.8, 32.7	39.8, 50 > 10.2; 1.1, 21.2 > 20.1, 30.3	Red Sugar in 5c.c
0, 50 >; 0, 30 >; 0, 87 >; 31.8; 181.8	0, 50 >; 0, 56 >; 0, 24.6 >; 124.6	0, 50 >; 0, 50 >; 0, 35 >; 135	35.3, 45.6 >; 10.3	0, 40.7 >; 40.7	0, 52.5 >; 0, 50 >; 21.3, 44.0 > 23.3; 102.5; 125.8	Total Sugar in 5c.c

VII	VII	VIII	IX	XI	XII	XIV	Culture no/.
0.7	20.2	40	40	2.4	22	35.1	Acid in 5c.c
20.2	40	19.8	47.6	22	35.1	alk	
20.2	19.8		7.6	19.8	13.1	-alk	
0	0		16.9	8.4	5.1	0	Total sugar in 5c.c
50	50		50	50	40.7	14.0	
0.6	0		33.1	41.6			
50	50		0	0			
0.3	0		50	50			
50	50		0	8.4			
0	0		8.4	5.1			
19	16.9		8.4	5.1			
16.9	166.9		91.5	46.7	35.6	14.0	
13.9	0		16.6	2.	23.8	16.2	Reducing sugar in 5c.c
37.9	16.6		50	23.8	50	22.4	
			33.4		26.2		
			0		0		
			2		16.2		
			2		16.2		
24.0	16.6		35.4	21.8	42.4	6.2	
0.6800	0.6740		0.6900	0.7100	0.6680	0.6886	Phenyl hydrazone
0.7080	0.6930		0.7270	0.7120	0.6700	0.7186	
0.0280	0.0390		0.0370	0.0020	0.0020	0.0300	Date 21/8/57

Rooms no 21 Sanskrit Dept.

16	23	25	
0.9 / 4.6 → 13.7	14.6 / 25 → 10.4	27.7 / 35.8 → 8.1	Acid in Sc.c
0 / 23.8 → 23.8	24.5 / 40 → 15.5	10 / 50 / 0 / 12.5 → 12.5	Red Sugar in Sc.c
		22.5	
12.5 / 50 → 37.5	10.4 / 50 → 39.6	3.5 / 3.10 → 27.5	Total Sugar in Sc.c
0 / 10.4 → 10.4	0 / 3.4 → 3.4		
47.9	43.0		
0.6810	0.6564	0.6934	Phenyl hydrazine
0.7300	0.7090	0.7360	
0.0490	0.0526	0.0426	

9	15	17	18	20	21	
		Na_2CO_3 N/50			Na_2CO_3 N/20	
7.6	0, 10.4	10.4, 34. → 23.6	3.5, 8.5	8.9, 14.6	14.6, 20.6	Total Acid in 5 c.c
	0, 50, 0, 35.2 → 85.2	0.7, 50 → 49.3; 0.0, 30.4 → 30.4; 79.7	0, 50, 0, 50, 0, 16.8 → 116.8	16.8, 50 → 33.2; 0, 50, 0, 2.3 → 50. + 2.3 = 85.5	2.3, 50 → 47.7; 0 → 31.4; 31.4 → 79.1	Red Sugar 5 c.c
	0.1, 50, 0, 50, 0.4, 14.4 → 114.4	0, 50, 0, 50, 0, 50 → 150	0, 50, 0, 50, 0, 50, 0, 22 → 150 + 22 = 172	0, 50, 0, 29.9 → 79.9	0 29.9, 50, 0, 50, 0, 16 → 116	Total Sugar 5 c.c.
0, 14.3 → 14.3	14.3, 45.4 → 31.1	4.4, 34.6 → 30.2	0.5, 13.1 → 12.6	13.1, 25.9 → 12.8	25.9, 32.6 → 6.7	Acid by oxidation Benzyle; 5 c.c dil in water titr X Na_2CO_3 N/2
N/50: 0, 2.9	2.9, 30.2 → 27.3	30.20, 50 → 19.8; 23.6, 30.8 → 7.2; 27.0	32.6, 37.8 → 5.2 + .1	37.8, 40.8 → 3.0	40.8, 43.6 → 2.8	Volatile acid in 5 c.c.
2.8						Volatile

Analysis of Sugar set. 13th Sept.

31.6
60.8
38.4

Bantanol cultures

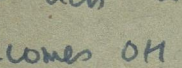
17	19	22	For 5 c.c.
-0-	2.3 / 2.9 > 0.6	2.9 / 3.8 > 0.9	Volatile acid
0.6 / 1.2 > 0.6	1.2 / 1.8 > 0.6	1.8 / 2.3 > 0.5	Total acid
0 / 23.4 > 23.4 !	23.4 / 50 / 26.6 > + 0 / 5.8 > 32.4	5.8 / 14.7 > 8.9	Red sugar
~~14.7 / 30.2 > 15.5~~ ! 0 / 40.1 > 40.1 ✓	~~30.2 / 50 / 19.8 > + 5.9 / 0 > 25.7~~ 0 / 47.6 >	5.9 / 45.6 > 39.7	Total sugar
3.9 / 8.9 > 6.0	8.9 / 17.5 / 8.4 > 9.0	17.5 / 27.1 > 9.6	Acid ~~[illegible]~~ ~~[illegible]~~

$N/20$ Na_2CO_3 used onwards.

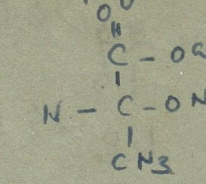
These are yet to be calculated.

Increase of reducing property :-

Other than formyl group, formate, lactate chloroform iodoform uric acid etc. Other compds can also reducing Fehling's solution. That is the following arrangement of hydrogen makes the H atom so labile that it acts as reducing group. (it catches one O and becomes OH group)



Formate



Lactate.

If a carbonyl or oxygen or ester group is attached to the C- carrying H atom., then hydrogen is reducing. But as ketene $CH_2=CO$ does not have such reducing property Carbonyl oxygen and alkyl attached to the carbon contg hydrogen makes an ideal aldehyde. 3 halogen atom weak ortho + para directing groups also make the hydrogen attached to the carbon atom reducing - OH & $-NH_2$ are strong o & p directing groups yet only one of such group, does not make the hydrogen atom sufficiently active as to act as reducing H. Of course the compounds contg 2 or more OH, NH_2 groups attached to one carbon atom are so unstable that their

31.3168
4.8
36.1168

31.31
5.6
36.91

.96×5 — ~~3.84~~ 4.80 citric acid in 250 c.c.

1.4×4 — 5.6 in 200 c.c 50+20 = 70 = 80
Sod. phos.

The effect of pH. on Aerobacter Aerogenes.

using sugar. (2)
8 cultures.

~~use phosphate buffer.~~

~~pH, 3, 3.5, 4, 4.5, 5, 5.5, 6, 7, 8, 9, 10~~

{citric acid / disod. phosphate} buffer. pH, 3. 4, 5, 5.6, 6, 6.6, 7, 8, ~~9, 10~~

5% sugar. and the other chemicals as in the case of antisep. etc. 200 c.c total volume

Seed culture 50 c.c.

Seed with Org. nos: 2.

Date of Seeding — 1/4/~~58~~

Date of analysis —

1	3
2	4
3	5
4 (not studied) 5.6	— X Inf.
5	6
6	6.6 — Inf. Sl.
7 (not studied.)	— X Inf.
8	8

Analysis of Seed culture: —

Total Sugar. ~~R. Sugar.~~
in 5 c.c.
12.4 c.c.

B ~~stained~~.

Five 800 c.c cultured media were prepared each contg

Sugar – 18 gm.

KH_2PO_4 = 0.9 gm

K_2HPO_4 = 2.1 gm

$(NH_4)_2SO_4$ = 1.02 gm

$MgSO_4$ – 0.48 gm.

The culture media were seeded with. Serial no/:

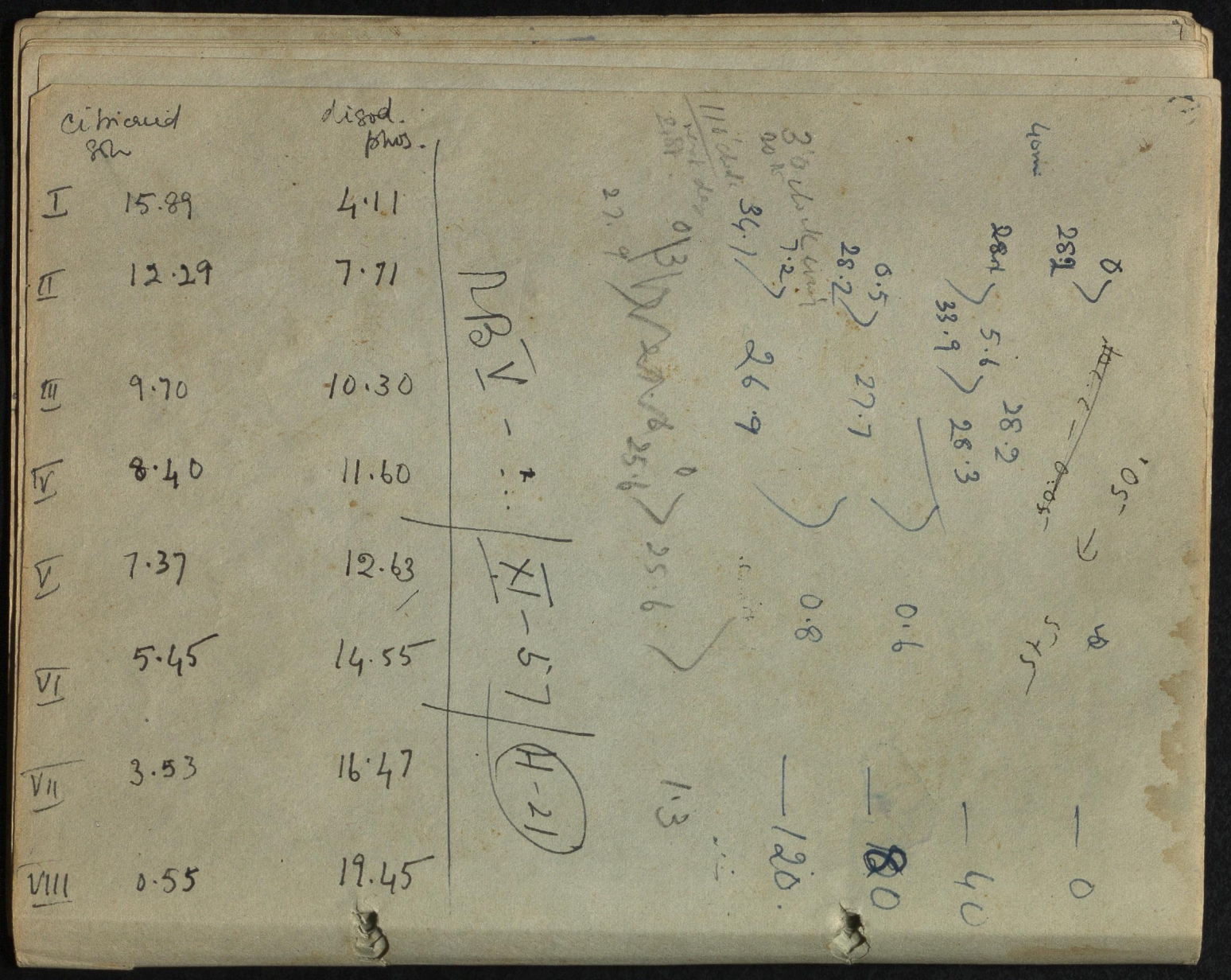
1) Aerobacter Aerogenes. (2)
2) Bacillus Subtilis (10)
3) Serratia Marcescens (8)
4) Aeromonas hydrophila (24)
5) Bacillus Polymyxa. (4)

After 15 days. these are to be analysed.

Date of seeding – 12.9.57.

The effect of the amount of seed culture on Aerobacter aerogenes. using ~~molasses~~ sugar.

	citricaid 8h	disod. phos.
I	15.89	4.11
II	12.29	7.71
III	9.70	10.30
IV	8.40	11.60
V	7.37	12.63
VI	5.45	14.55
VII	3.53	16.47
VIII	0.55	19.45



molasses (1)

1) Set up F2 cultures conty 5 per cent sugar
Analyse after one days, 2 days & 3 days.
For seeding each set of 3 cultures use the following four organisms.

2) Use diffr concns of phosphate with and without $CaCO_3$
use 25 c.c of seed culture

$CaCO_3$	$mCaCO_3$	Two cultures. one with $CaCO_3$ no phos. one without $CaCO_3$ no phos.
5 c.c phos	→ 1	
10 c.c phos	→ 2	
15 c.c phos	→ 3	

with

3] Study change in concentration.
Seed Culture – 25 c.c in a 100 c.c culture.

4] Change in amount of seed culture
25 c.c, 50 c.c and 75 c.c.

1. Concn.
2. Phosphate & Carbonate
3. Seed.
4. Time
5. pH.

6.4 + 15.9 → 16.3
16.3 + 12.3 → 28.1
28.6 + 9.7 → 38.3
38.3 + 8.4 → 46.7
46.7 → 7.4

7.4 + 5.45 → 12.85
12.85 + 3.5 → 16.35
16.35 + 0.55 → 16.90

4.1
11.8 +14.55 → 31.05
22.1 +19.45 → 50.5
33.7 26.2 45.15
46.3

(2)

Study of the effect of change in concentration

First molasses was diluted four times (100 ⇒ 400 c.c) and six cultures were prepared each containing the following.

	Molasses soln.	water	seed culture
I	~~25~~ c.c.	125 c.c	~~50~~ c.c
II	~~250~~ c.c	100 c.c	"
III	~~75~~ ~~125~~ c.c	75 c.c	50 c.c broken
IV	~~100~~ c.c	~~40~~ 50+90 c.c	50 c.c (6)
V	125 c.c	25 c.c.	50 c.c
VI	~~150~~ c.c	25 c.c	~~50~~ c.c. 25 c.c.
~~VII~~	[Control] ~~50 c.c~~ 10 c.c	~~150~~ c.c 90 c.c	nil. (7)

Seeded on 18th Sept. pH 5.6

③ Seed Culture analysis results {12.9.57 / 18.9.57}

	Red sugar	Total sugar	b d r
alkaline.	30.3 / 42.2 } 11.9	0 / 30.2 } 30.2.	0.65 20
			0.6792
			0.0272

Organism no: II Analysed on 4/10/57

pH 6.4 Seeded on 21st Sept '57.

Contents:—	Molasses	water	Seed culture.	
1)	25 c.c.	125	50 c.c.	
2)	50	100	"	
3)	75	75	"	
4)	100	50	"	
5)	125	25	"	not growing.
6)	150	—	"	
Control.	50	150.		

(2)

Seed Culture Analysis shows:— {12.9.57 / 21.9.57}

Acidity.	Red Sugar	Total Sugar	b d r.
alkaline.	1.5 / 9.4 } X9		0.6680
			0.6826
	9.4		0.0146
	19.5 } X1		
	10.1		

III Phosphate & carbonate. pH 6.3

org no: II.

(4) Concentration used in

50 c.c mixture — 100 c.c water (less in 4th series) — 50 c.c seed culture.

Phosphate solution prepared:-

I	II	III	
nil -	5 c.c.	10 c.c.	no $CaCO_3$
IV	V	VI	
nil -	5 c.c	10 c.c.	with 2g. $CaCO_3$.

Control culture also to be prepared. Find amount of phos while analysing.

Cultures seeded on ___

Prepare a seed culture. Contg. 400 c.c

Sugar -	3%	12
KH_2PO_4 -	0.15	0.60
K_2HPO_4 -	0.35	1.40
$MgSO_4$ -	0.08	0.32
$(NH_4)_2SO_4$ -	0.17	0.68

Seeded on. 28/9/57

Control — not seeded pH 6.3

1.— $CaCO_3$ — 2 gm.

2- $CuSO_4$ 0.02 gm in 200 c.c

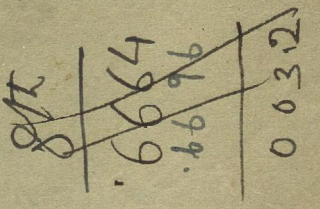
3 — Phosphate — 0.25gm total

4- $ZnSO_4$ — 0.02

5 — Nickel ~~Sulphate~~ Chloride — 0.02

6 - Milk — 2 c.c.

7 Molasses only —



Concentration

50 c.c molasses. 100 c.c water. 50 c.c Seed Culture

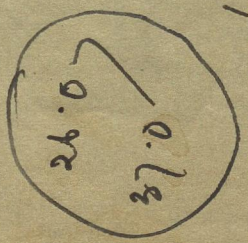
Analysed 5/10/57

Cultures seeded on — 28/9/57

Seed Culture Analysis shows:— Seeded on — 18/9/57
Analysed on ~~28/9/57~~

Oil

Acid. Red sugar 11.0 Total sugar 19.2



23.6
42.8

0.6386
0.6630

0.0244

(6)

Acetaldehyde

malic acid $COOH - CHOH - CH_2 . COOH$

Tartaric + Acetic acid

glycerine

2% of these with sugar.

Study the yield using them separately

The sugar titration shows reducing non inverted contents ($K_2Cr_2O_7$ - 9.8008 gm/l)

Analysis of Cultures I concentration. 4/10/57

(1)	(2)	(3)	(4)	(5)	(6)	(7)	
0 → 0·6	·6 → 1·0	1·6 → 2·1	4·5 → 2·1	6·6 → 1·1	7·7 → 0·5	8·2 → 0·8	5 c.c. Acid in 5 c.c
0·6	1·6	3·7	6·6	7·7	8·2	9·0	
0 → 98·3	0 → 1·0 → 44·2 → 43·2	0 → 95·0	0 → 49·5	0 → 33·2	0 → 5·9 → 27·9 → 42·0	0 → 21·9	Red Sugar in 5 c.c
0·3 → 15·9 → 15·6	15·9 → 43·0 → 27·1	Total c.c required 68·2 c.c.	0 → 41·5	0 → 44·6	4·9 → 8·5 → 3·6	8·8 → 23·2 → 14·4	Total Sugar in 5 c.c
0·6726	0·6500	0·6520	0·6762	0·6462	0·6290	0·6568	
0·6880	0·6740	0·6845	0·7190	0·6820	0·6590	0·6710	
0·0154	·0240	·0335	·0428	0·0358	·0300	0·0142	Potassium dichromate

(1)

5/10/57

1 Acid 2 3 4 5 6 7 8

1·9 + 11·5 + 13·5 → 26 → 3·9

D → 1·8 → 1·8 → 1·7 → 3·3 → 1·7 → 5·5 → 1·2

1·8 3·5 5·2 6·7

7·4 → 8·8 → 9 → 1·4 → 10·3 → 1·3 → 10·3 → 10·7 → 0·4

Acid. 5 c.c. filled with Na_2CO_3

0 → 1·8 → 1·8 → 8·8 → 10·7 → 19·6 → 8·9 → 38

1·8 10·6 19·6

21·0 → 0 → 22·0 → 0 → 47·5 → 23·5 → 49·8 → 15·6 → 34·2 → 0 → 3·8

38·4 70·0 15·6

21 → 41·8 → 20·8 → 3·9 → 12·1 → 9·2

dichromate N/5

Red Sugar in sc.c.

Total Sugar in sc.c.

I Concentrated solution II 0 III 5·8 IV 0 V 15·6 VI 2 VII

13·3 → 33·0 → 19·7 → 22 → 22 → 39·5 → 33·7 → 15·6 → 15·6 → 31·5 → 15·9 → 32·2 → 49·5 → 17·3 → 16·6 → 16·6 → 10·6 → 50 → 39·4

Red Sugar in sc.c.

0·6490	0·6470	0·6610	0·6642	0·6786	0·6690	0·6390	0·6700
0·6920	0·6946	0·7080	0·7390	0·7414	0·7360	0·6915	0·7062
·0430	0·0476	·0470	·0748	·0628	·0670	·0525	·0362

Butane diol

(20)

Prepare a seed Culture containing

Sugar – 12 g.

KH_2PO_4 – 0.60 g

K_2HPO_4 – 1.40 g

$MgSO_4$ – 0.32 g

$(NH_4)_2SO_4$ – 0.68 g.

Volume – 400 c.c.

Seeded with org. no/: 4 on 14/10/57.

Prepare molasses solution. pH — 6.4

Concentration as follows.

Molasses	Water.	Seed Culture
50 c.c	100 c.c	50 c.c.

Add to

I Acetaldehyde 4 c.c

II Malic acid 4 gms

III ~~Natte~~ $CaCO_3$ 2g - HAc 4 c.c

IV glycerine 4 c.c. 4 gms.

V only molasses

VI glyceraldehyde glycerine 4 c.c. H_2O_2

VII. Control.

The cultures are seeded on — 14/10/57.

Analysis of the seed (11) culture seeded on 28/9/57.

Date of analysis.

Acid.	Red sugar. 5c.c	Total sugar 5c.c.	B. did in 5c.c.

See page (15)

Seeded with organism no/: 4. on 14/10/57.

culture no/: 4 analysed on 26/10/57 page 16

Analysed on — 28/10/57 Page no/: (17)

Before analysis prepare the following:—

Fehling solution no/: 1.

no/: 2.

[N/5 dichromate solution:]

Standardise this solution with standard glucose solution.

Red sugar to be estimated by direct titration

So 6, 50 c.c flasks are required.

Try no dilution:—

Room temp

✓

(12) 10 mg wo/: 2

3 conc. $ZnSO_4$ ·01 ·02 ·03

Prepare 6 Cultures containing the following.

1) $ZnSO_4$ + milk 0.02 + 2c.c 50c.c molasses. – (infected)

2) $ZnSO_4$ + Nichloride 0.02 + 0.02

3) $ZnSO_4$ + Nichloride + milk. 0.02 + 0.02 + 2c.c.

4) Nichloride + milk. 0.02 + 2c.c.

5) Molasses only.

6) control. ✓

Seeded on — 26/10/57

Seed Culture analysis { Seeded on – 16.10.57 / Analysed on – 26.10.57 }

Acid.	Red Sugar	Total Sugar.	Butane diol	} in 5c.c
0.4 c.c. 18/20 Na_2CO_3	no red Sugar	30.0 / 48.3 } 18.3	0.6610 / 0.6700 / 0·0090	

Analysed on — 11/11/57.

Room temp.

(13)

Prepare six cultures containing the following org n-1:2

$ZnSO_4$	0.01	—	(1)
	0.02	—	(2)
	0.03	—	(3)
	0.04	—	(4)
Molasses only		—	(5)
— Control.		—	(6).
$ZnSO_4$ 0.02 + milk			(7)

Dissolve 1gm of $ZnSO_4$ in 100c.c.

Add 1c.c, 2c.c., 3c.c & 4c.c

&

Seeded on 2c.c. — 12-11-57.

Seed culture analysis { Seeded on 26.10.57, Analysed on 12.11.57 }

50c.c. molasses + 100c.c. water. + 50c.c. seed culture.

Seed culture analysis:— on:-

Acid.	Red. Sugar.	Tot. sugar.	Butane diol } in 5c.c
0.6 c.c.	23.1, 43.6) 20.5	0, 21.6) 21.6.	0.6492
			0.6820
			0.0328

dichro met.

Two seed cultures to be prepd.

Seeded on — 12/11/57.

Incubation.

$21.6 \times .028$

1728
432
.6048

$.032 \times 1.01$

032
3200
.03232

18
27
45

(14)

Repeat concentration cultures. with Org. no/: 2
with org no/: 4

Molasses	Molasses		Water	Seed
				50 also.
10 c.c	10	+	140	
25 c.c	25	–	125	
50 c.c	~~30~~	~~–~~	~~120~~	
75 c.c	50	–	100	
100 c.c.	75	–	75	
Control.	100	–	50	

Control containing 50 c.c molasses.
~~... is sufficient~~.

Room temp 12 Cultures. (The set of no/: 4 got infected).
These are seeded on – 26/11/57
Analysed on –

Acid 0.9

Analysis of seed culture 2

5 cc R.S. T.S. – Balance

Seeded on. 12/11/57
Analysed 28/11/57

0 c.c } 46.1

Total
0.6420
0.6820
0.0400

46.1 × 10 × .0028 × 2
46.1 × .028
3.688 1.2908

Seeded on 14/10/57
Culture 4
Analysed – 28/11/57

Acid 0.5 c.c
RS TS

21 } 26.2

0.6340
0.6820

.044

.04 × 1.01
.0404

Effect of concentration. Org. no: 4.

	Molasses	water	Seed culture
I	10 c.c	140	50
II	25 c.c	125	50 ✓
III	50 c.c	100	50
IV	75 c.c.	75	——— (Infected)
V	100 c.c	50	
VI	Control conty 50 c.c	150.	—

Analysis of seed culture: 15/10/57 (Same for 1/5 dilution media) { Seeded on 12/9/57 } (for page no: 10 set)

Room temp.

Acid.		Red sugar.		Total sugar		Butan diol
20.7	0.4	33.7	6.1	89.8	10.5	0.6430
21.1		39.8		50.3		0.7570
						0.1140

Seed culture was also prepared

Seeded on — 15/10/57

Analysed on — 4/11/57

Org no 4

Prepare 6 cultures containing

1) 1% glg
2) 2%
3) 4%
4) 6%
5) 5% Sucrose

Make a solution of following minerals

$CaCO_3$ - 0.2g
$MgCO_3$ - 0.2
NaH_2PO_4 - 0.2
K_2SO_4 - 0.2
NaCl - 0.2
& 2gms of Amm. Sulphate

Digest with HCl. filter and ~~add 50 c.c~~ make upto 400 c.c. Add 50 c.c of this in each culture of total volume 150 c.c pH should be 6.5

Seed them with 25 c.c of seed culture from glycerine culture on page (10)

The cultures were seeded on (26/10/57)

Analysis of glycerine culture (4)

Acid.	Hcl Sugar in 5 c.c		Total sugar in 5 c.c		Butane diol
5 c.c					
alk.	0	7.4	7.4	22.6	0.6670
	7.4		30.0		0.7210
			dichromate		0.0540

Analysed on 12/12/57

4
30
45 days

29/10/57.

1 ①	2 ②	3 ③	4 ⑤	5 ⑥	6 ⑦ control.	
0.1, 0.9 > 0.8	1.0, 12.8 > 11.8	12.8 > alk	alk	12.8 > alk	alk.	Acid
0, 2.8 > 2.8	0.5, 1.7 > 1.2	0.0, 2.3 > 2.3	0.1, 13.2 > 13.1	0, 18.5 > 18.5	0, 11.6 > 11.6	
			diluted 25 → 100 c.c			
0, 32.5 >	32.5, 50 > 17.5; + 0.1, 25.2 > 25.1	25.2, 50 + > 27.8; 0, 6.1 > 6.1	6.1, 36.0 >	36.0, 50 > 14.0; + 0, 28.8 > 28.8	28.8, 50 + 0, 21.4 > 21.2	
32.5	42.6	33.9	29.9	42.8	21.4	42.6
0.6738	0.6356	0.6450	0.6250	0.6530	0.6644	
0.7300	0.6560	0.6600	0.6940	0.6680	0.6690	
0.0562	.0214	.0250	.0690	.0150	.0054	

Methylene blue indicator
Red sugar titrating without dilution against fehling

75 k 1 g/l (dichromate N/5)

Aceta.
0.7124
0.8130
0.1006

Butane diol.

---

⑧ Strength of fehling used here: → (estimate)

5 c.c A + 5 c.c B = 4.6 c.c 1 g of ~~sugar~~ sucrose in 100 c.c
= 0.~~0046~~ .046

Concentration Cultures of no: 4 Date 4/11/57. 60

I	II	III	IV	V
33.5 / 33.8 → 6.3	33.8 / 34.0 → 0.2	~~34.2~~ alk. →	alk	alk
nodules				
0.2 / 19.4 → 19.2	0.1 / 22.3 → 22.2	0 / 9.0 → 9.0	0.4 / 5.0 → 4.6	0 / 10c.c → 10c.c dichromate
0 / 6.0 → 6.0	6.0 / 23.0 → 17.0	23.1 / 50 → 26.9; 0.0 / 10.5 → 10.5; 37.4	10.9 / 50 → 39.1; 0.0 / 14.2 → 14.2; 53.3	14.2 / 40.5 → 26.3
0.6380 / 0.7000 → 0.0620	0.6340 / 0.7040 → 0.0700	0.6490 / 0.6664 → 0.0174	0.6400 / 0.6984 → .0584	0.6930 / 0.6984 → 0.0054

Acid in 5c.c N/20 $KMnO_4$

~~Total~~ Reducing sugar in 5c.c

Red sugar 25c.c → 100c.c Titr. against Fehlings

Butane diol.

Curbine II

Red 10c. → 100 c.c × 5 c.c + 5 c.c. Fehling = 44·4 c.c.

Total " " = 32.

14. 2. 58, 3 P.M.

Ca P

11·308

21 hrs Comp clotted. 1. Papain – Cu 1st fraction – clotted in 15 / 2 / 35 10·30

25 hrs 2. Papain – 2nd fraction – ~~62·173~~ clotted in " 25/2/58. ~~27·185~~ 23·274.

✓ 3. Papain Ca fraction. – unclotted on 17th 16·76 % Ca

21 hrs Comp clotted 4. Fermented papain 1st fraction. – clotted. 27·155.

5 Fermentin – papain 2nd fraction. unclotted on 17th.

✓ 6. Ferm papain – Ca fraction. unclotted on 17th 13·21 Ca ?

clotted 25 hrs 7. Papain – C

✓ 8 – Milk. unclotted on 25th. 13·21

Analysis of cultures on page no/: (12)   Date 11/11/57

5 c.c. Acidity		Red Sugar 25 → 100 c.c. (Methylene blue)		Total Sugar in 5 c.c. against dichromate			
1	2.1 / 3.0 → 0.9	0 c.c. / 10.3 →	10.3	0 / 50 c.c. →	50 c.c.	0.6620 / 0.6700	0.0080
2,	3.35 / 3.6 → 0.25	0 c.c / 10.5 →	10.5	0 / 23.6 →	23.6 c.c.	0.6600 / 0.6736	·0136
3	4.7 / 5.5 → 0.8	0 c.c. / 10.5 →	10.5	23.6 / 50 → ; + 0 / 14.8 →	26.4 + 14.8 = 41.2	0.6516 / 0.7074	0.0558
4	5.5 / 6.0 ; 6. / 6.3 → 0.3	0 c.c / 11 c.c →	11 c.c	16.9 / 50 → ; 0 / 7.5 →	33.1 + 7.5 = 40.6	0.6510 / 0.6890	0.0380
5.	5.5 / 6.0 → 0.5	0 / 10.1 →	10.1 c.c.	7.5 / 23.1 →	15.6	0.6350 / 0.6370	0.0020

34.5 / 33.2

0.4 / 2.3 / 2.7

28

21 | Glycerine Culture page 16. Total volume - 225 c.c
Date. 12/12/57

	Analysis Butane diol in 5 c.c.	Total sugar present in 5 c.c.	Red. sugar present in 5 c.c	Acid in 5 c.c.
I B	0.6650 X	21.1 → 32.2 → 1.1	0 → 3.95 → 3.95	0 → .45 → 4.5
III B	0.6670 X	22.25 → 24.7 → 2.45	3.95 → 6.4 → 2.45	.45 → .8 → 3.5
II B	0.6590 X	25.9 → 26.8 → 0.9	6.4 → 8.85 → 2.45	8 → 1.15 → 3.5
IV B	0.6600 V	26.8 → 27.6 → 0.8	8.85 → 11.4 → 2.55	1.2 → 1.45 → 2.5
		27.6 → 41.4 → 13.8	11.4 → 21.1 → 9.9	1.45 → 1.90 →
V B	0.6740 − 0.6680 = 0.0060			

Repeated.
IV R 42.4 → 48.2 → 5.8

I X	II	III	IV	V	IV T	
.6846	.6446	.6638	.6528	.6696	0.0 → 6.2 → 6.2	
		.6550		.6730	V R	6.2 → 18.9 → 13.7
				.0034	V Total	18.9 → 29.0 → 10.1

I H X	II H X	III H X	IV H X	V H	
0.6320	0.6540	0.6480	0.6730	0.6560	29.4 → 32.2 → 2.8
				0.6650 IV to be	
				0.0090	

I D X	II D X	III D X	IV D X	V D X
0.6750	0.6638	0.6562	0.6654	0.6252

221 Concentration cultures of org. no 1 : 2. Page 14.

$F_1 + F_2$ requires 4.9 c.c of 1gm in 100 c.c. 5/1/58
5 c.c + 5 c.c = .049 gm of sugar
Analysing Date. 7/1/58

Buffer 5 c.c	Red Sugar in 25 c.c. dil 100 c.c.		Total Sugar in 5 c.c.		Butane dil.	①
alk	0.1 / 14.3	14.3	21 c.c.	I B		1.1350 1
alk	0 / 20.6	20.6	29 + 41 = 33.1	II B		1.1300 2
0.7	0 / 10.5	10.5	52.6	III B		1.1600 3
32.5	0 / 6.7	6.7 — 73.8		IV B		1.1520 4
117.0	0 / 5.1	5.1 — 80.4		V B		1.1720 5
alk.	0 / 10.4	10.4 — 43.5		VI B.		1.1530 6

10	25	50	100	100	
I H	II H	III H	IV H	V H	VI H
1.2180	1.2080	1.2010	1.1680	1.1550	1.1730
1.2050 X	1.2160	1.2346	1.1942	1.2078	1.1900
	.008	.0336	.0262	.0528	.017

I B 1.1970 — 1.2350 — .0380 — I D

II B 1.2118 — 1.2178 — .0060 — II D

III B 1.2060 — 1.2250 — .0190 — III D

IV B 1.2128 — 1.2512 — .0384 — IV D

V B 1.2224 — 1.2534 — .0290 — V D

VI B 1.2236 — 1.2536 — VI D

I D 1.2170 — 1.2446 — .0276

II D 1.2180 — 1.2060 — .0120

1.1790 — 1.2186 — .0396

1.1772 — 1.1946 — .0174

1.1914 — 1.1818 — .0096

1.1760 — 1.1800 — .0040

Effect of ZnSO4 on org no 1: 2

Date of analysis:- 2/1/58
~~27/12/57~~

	Acid in 5 c.c.	Methylene blue Reducing sol. in dilution 25→100 c.c. (5 c.c N + 5 c.c B)	Total Sugar 5 c.c. x dichromate N/5	Butane acid.	Distillate
1	0.1 / 6.1	6.0	57.1 ~~2~~ — 57.1	I B 1.170	I D 1.1180
2	0.0 / 6.6	6.6	52.0	II B 1.1950	II D 1.1330
3	0.5 / 7.6	7.1	51.2	III B 1.22.	III D 1.1260
4	0.3 / 6.7	6.4	40.8	IV B 1.1618	IV D 1.1466
5	0.1 / 1.7	6.6	52.9	V B 1.1380	V D 1.1440
6	0.2 / 10.3	10.1	40.3	VI B 1.1340	VI D 1.1460
7 alk.	0.0 / 6.4	6.4	43.0	VII B. 1.116	VII D 1.1300

I	II	III	IV	V	VI	VII
1.15	1.157	1.162	1.162	1.17	1.13	1.14

Perform the same set with sucrose culture

4/1/58.

I H	II H	III H	IV H	V H	VI H	VII H
1.1730	1.1550	1.168	1.164	1.162	1.153	1.165

negative results.

Concentration Culture for org. no 1: 4

Molasses	Water	Seed
10 c.c.	140 c.c.	50 c.c
25	125	"
50	100	"
75	75	"
100	50	"
Control 50.	150	—

Room temp. Seeded on — 14/12/57

Seed Culture Analysis — { Date Seeding :— 26/11/57 / Analysis :— 16/12/57

R. Sugar.
0.2
25.0 } 27.8

Acid 5 c.c
0.6 c.c
Molarity

Total Sugar.
28.0 } 22
50 +
33.2
39.0 } 5.8
27.8

Butane diol
0.6790
X

27.8 × 10 × .028
27.8 × .028 6
22 2/5 6 4 .778 4

Prepare a Seed Culture also.

Results of analysis of circum. Cultures:- Page 24. 23/1/58.

	Acid c.c	Red. Sugar 25 → 100 c.c.	Total Sugar. 23/1/58.		Date Analysis	
				8X	XH	
1	0.3 c.c	21.7 c.c	0.3 15.2 → 14.9	1.1630 1.1636 → 0.006	1.1430 1.1450 → 0.002	
2	0.6 c.c	7.9 c.c	15.2 49.5 → 34.3	1.1470 1.1490 → 0.0020	1.1750 1.1810 → 0.0060	
3	4.6 c.c	5.3 c.c	0.2 44.8 → 44.6	1.1180 1.1340 → 0.0160	1.1600 1.1800 → 0.02	
4	86.5 c.c	4.2 c.c	0.2 50 → 49.8 0.1 9.7 → 9.6 59.4	1.1540 1.1780 → 0.0240	1.1470 ~~1.1600~~ 1.1700 → 0.0230	
5	150 c.c.	3.2 c.c.	12.3 50 → 37.7 0.1 33.6 → 33.5 71.2	1.1940 1.2284 → 0.0344	1.1240 ~~1.1590~~ 0.0350	
6	alk.	10.6 c.c.	33.6 50 → 16.4 +0.05 19.45 → 19.4 35.8	1.1440 1.1529 → 0.0082	1.1210 1.1340 → 0.0130	

XD: 1.1180. 1.1130 | 1.1420 | 1.1500 | 1.1880 | 1.1710 1.1720 | 1.1710

XB:

IB	IIB	IIIB	IIIB	IVB	VB
1.1674	1.1620	1.1690	1.1500	0.6520	
1.1568	1.1620	1.1736	1.1350	0.6470	
1.1600	1.1650	1.1790	1.1440	0.6520	
nil	0.003	0.0100	nil	nil	

26

Prepare 5 Khar yeast cultures. (400 c.c)

each containing.

Sod. phosphate – 0·2
$CaCO_3$ – 0·2
$MgCO_3$ – 0·2
NaCl – 0·2
K_2SO_4 – 0·2
$(NH_4)_2SO_4$ – 2 gm
Sugar – 5 gms.
350 c.c water.

Make a 5% of $ZnSO_4$
1 g in 100 c.c.
Add – 0 c.c
– 5 c.c
– 10 c.c
– 15 c.c
& – 20 c.c.

+ $ZnSO_4$ 0·00, 0·05 0·10 0·15 & 0·20 in 400 c.c.

Seed with 50 c.c. of Khar yeast.

Analysis Analyse after 15 days.

Seeded on 20/1/58.

Analysis. Total Sugar. Reseeded on 7/2/58 Butanediol. 7/2/58

38·7 c.c 6 0·6800 15
diluted 38·7 × ·028 0·6740 22/2/58
for 30·96 1·0636
5 c.c.

Seed Culture Analysis in 5 c.c

T. S. Butanediol.

3 do 48 c.c VIII × 1·1630
487·028
204
112
1324 28 c.c. IX × 1·1610
× ·028
32·784

I no II Seeded 12/11/57 Anal 3/1/58
II no 8 Seeded 20/12/57 Anal 3/1/58

Serratia Marcescens cultures. pH - 6.

Sucrose – 5 % 10 g
$MgSO_4$ – 0.08 % .16
$(NH_4)_2SO_4$ – 0.17 % .34
Phosphate – 0.3 (mixture) [KH_2PO_4 0.15 + K_2HPO_4 0.35]
milk – 0 c.c. 1 c.c & 2 c.c.
per 100 c.c.

200 c.c Seed with 50 c.c. seed culture.
Seeded on 2/1/58

Reducing capacity

A. Aerogenes cultures. ~~Seeded on 7/2/58~~

Sucrose – 5 % 10 g
$MgSO_4$ – 0.08 % 0.16
$(NH_4)_2SO_4$ – 0.17 % 0.34
① Phosphate – 0.3 % only. [0.15 + 0.35]
② with 1 % $CaCO_3$ also 2 g.
③ with 0.01 % $CuSO_4$ also (& no $CaCO_3$) 0.02.

150 c.c wash. Seed with 50 c.c. seed culture
Seeded on 2/1/58.

1.5 g KH_2PO_4 } in 100 c.c. 2.25 g
3.5 g K_2HPO_4 } 5.25
20 c.c added to each culture.

.3 3.5 1.75

28 Analysis of Dhar yeast culture 24/2/58

inc. — 5/2/58.

	Acid formed N/20 alk	Red Sugar	Total sugar.	Left.		Butane diol with Fe
0.1 / 1.6	1.5	21.6	21.6	12.1 / 23.7	11.6	—
1.6 / 3.1	1.5	20.7	20.7	11.4		—
3.1 / 4.35	1.25	19.6	19.6	24.1 / 35.9	11.8	—
4.35 / 5.8	1.45		19.9	12.3		
5.8 / 7.3	1.5	19.9	17.8	12.3		
		17.8		12.4 / 23.8	11.0	—

	D 1	D 2	D 3	D 4	D 5
Butane diol with hydrogen	1.1150	1.1370	1.1320	1.1450	1.1340
	1.1208	1.1436	1.1408	1.1546	1.1386
	.0058	.0066	.0088	.0096	.0046

1 Acid formed.	2 Red Sugar. 25 → 100 c.c.	3 Total Sugar.	4A. 5 c.c soln + Dr. ph. boil, filter dry + weigh.	4B (H) 5 c.c + 15 c.c water, distilled in dr ph let stand + weigh	4C (D) 5 c.c + dil HCl hydrolyse. add dr ph. let stand, filter dry & weigh	4D (Bycto neural procedure for titration with 5 c.c
1	30.6.	52.7	1.1685 .0021 1.1706	× 1.1750	× 1.2180	1.18743 .0006 1.18800
2 alkaline	6.7	44.2	1.1790 1.1826 0.0036	1.1450 1.1540 0.0100	1.2010 1.2100 0.0090	1.1980 1.2228 0.0248
3	6.3	45.2	1.1990 1.2080 0.0090	1.1620 1.1730 .0110	1.2080 1.2030 .0030	1.1920 1.2260 0.0340
4	3.4	61.8	1.1770 1.1820 0.0050	1.1690 1.1744 .0054	1.2040 1.2140 .0100	1.1296 1.1610 0.0316
5 alk alinic	0 → 50 → 26.6 → 50 → 32.6 → 50 → direct titr no dilu. 26.8	39.6	1.1900 1.1958 .0058	1.1470 1.1600 .0130	1.1970 1.2010 0.0040	1.1156 1.1450 0.0294
6	45 c.c.	47.2	1.2150 1.2304 .0154	1.1880 1.2050 0.0170	1.2096 1.2350 0.0254	1.1130 1.1430 0.0300

Analysis of S.M. cultures (page 27)

Analysis of A. aerogenes culture (2)

(3)

(25)

With sugar perform expts with
change in pH
in seed. quantity.

Analysis of other yeast cultures — 14/3/58 (today)

	I	II	III	IV	V
Acid in c.c.	0 → 2.05 → 2.05	2.05 → 4.1 → 2.05	4.1 → 6.1 → 2.0	6.1 → 8.1 → 2.0	8.2 → 10.35 → 2.15
25 → 50 c.c	10.5 → 34.5 → 24.0	6.4 → 29.0 → 22.6	7.9 → 26.2 → 18.3	6.7 → 28.3 → 21.6	6.1 → 25.7 → 19.6
			26.8 → 45.0 → 18.1	29.7 → 49.8 → 20.1	25.7 → 44.5 → 18.8
	24.0	22.6	18.1	20.1	18.8
Total sugar	13.7 → 20.0 → 6.3	21.2 → 27.8 → 6.6	6.3 → 13.7 → 7.4	0 → 6.3 → 6.3	27.8 → 35.6 → 7.8

$K_2Cr_2O_7$ sol. req.

Butane as dil. pri salt — nil

as hydrazone: D_1 0.6682 | D_2 ~~.6234~~ ~~.63~~ 1.6234. | D_3 0.6250 | D_4 .6700 | D_5 0.6234 | .6544

nil | nil | .7024 | 0.6456

0.0314 | 0.02229

23/12/57

Study of the ~~maleic~~ malic acid reduction system in B. polymyxa.

Make a solution as follows:

malic acid —	~~5 gm.~~ ~~2 gm.~~ 4 gm.
Sucrose —	~~0.5 gm.~~ 1 gm.
Tap water —	100 c.c.

Take ~~this solution~~ 10 c.c. of 6 test tubes cotton plug them and sterilise once at 15 lb pressure for 30 minutes. ~~F~~ P

Prepare three more sets as above containing ~~1 gm.~~ containing diff't quantities of sucrose. i.e. containing 2 gm. 4 gm. ~~of sucrose per 100~~ and 8 gm. of sucrose per 100 c.c. of the culture and prepare six 10 c.c. culture of each in diff't test tubes. and sterilised as above [use the set of the solution ~~for~~ ~~find out the pH~~ after adding 1/2 gm. ~~...~~ 36 tubes] with 5 c.c.

Seed all the above ~~...~~ cultures and ~~one~~ titrate of B. polymyxa cultures and ~~one~~ titrate one culture of each type with standard alkali using some suitable indicator.

Keep the rest 5 ~~...~~ culture of each set in an incubator at constant temp. (Keeping of the temp. constant) is essential.

~~Analyse as~~ Titrate with the same alkali as above ~~as follows~~ after 20 hrs. interval.

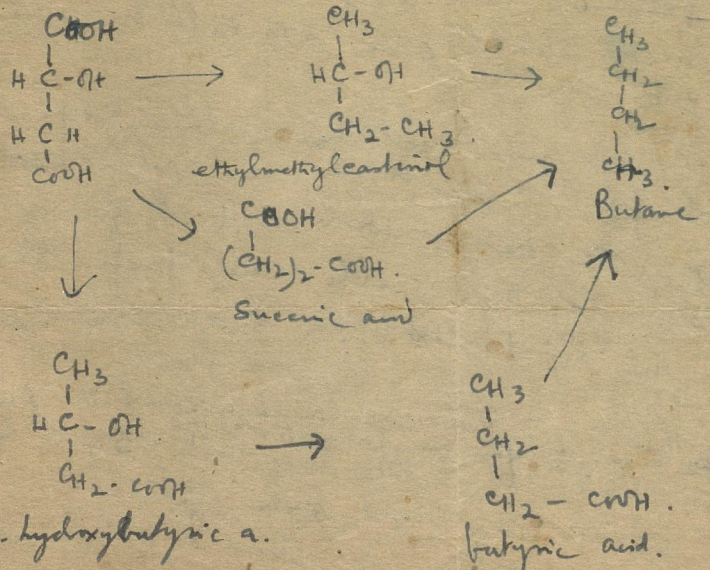
~~Seed~~

Repeat this experiments with tartaric acid & succinic acid.

Then an effort should be made to identify the products formed from the reaction under optimum conditions. The reduction can follow any of the following lines.

(a) Reduction of COOH group.

(b) Reduction of OH group.



In your previous experiments the malic acid was used only 0.02 gm. eqv. of acid was left whereas amounts to only 1 g/l. of the malic acid whereas the cultures you had prepared contained 20 g/l.

Rg 4/1/58
13.3

5 c.c of
from

23/12/57

Malic acid	4g	4g	4g	4g
Sucrose	1g	2g	4g	8g.

Add 100 c.c. tap water to each.

100 c.c taken in 7 test tubes each.

+4 c.c. seed culture (A. Aerogenes no 1: 2)

+1 c.c toluene.

1st set of readings. 23rd 2-30 P.M.

I	II	III	IV
0.1	8.8	13.0	15.1
50	50	50.0	50
0	0	0.0	0
8.4	13.0	14.6	15.6
58.3	41.2 + 13.0 = 54.2	51.6 + 1. = 52.6	50.9
	54.7	16.0 17.0	16.0
			0.2
			51.1

The flask cultures were kept in an incubator at 38°C. 58.3 54.7 52.6 50.9 51.1

Titrated with phenolphthalein indicator. Strength

	I	II	III	IV	
24th	17.3	17.3	17.2	16.7	13.3 + 30.0 = 16.7
25th	17.3	17.3	17.2	16.5	
26th	17.3	17.1	17.1	16.6	
27th	17.25	17.1	17.2	16.6	
				16.7	

Repeat without toluene

Malic acid	4g	4g	4g	4g
Sucrose	1g	2g.	4g	8g

Add 100 c.c tap water to each.

Take 10 c.c of this solution + 5 c.c of seed culture of (A. Aerogenes. NO/:2) {7 test tubes each}

Titrate after every 24 hours against caustic soda solution. {strength —}

Date.	I	II	III	IV

~~Prepare an N solution of Na_2CO_3.~~

	①	②	③
Malic Acid.	~~4g~~	~~8g~~	~~16g~~
Sucrose	0	4g	8g ~~16g~~

Solutions made up to 100. 5 c.c of this taken.

~~2%~~ 4% Solutions of malic acid, tartaric acid succinic acid.

Take 5 c.c. of the culture and 5 c.c of the solution. Keep 8 tubes of each. Keep at room temperature and titrate every 2 days.

Organisms. ~~Aerobacter Aerogenes 2.~~
Bacillus Polymyxa. 4.

To estimate the amt of acid present initially take zero reading. A solution of NaOH was prepared (To be standardised)

Make 2 litres culture ② of Bacillus ~~Subtilis~~ Polymyxa ④ ~~5~~ ⑩

Date — 5/2/58

From this the enzyme has to be separated.

	M_1	M_2	M_3	S_1	S_2	S_3	T_1	T_2	T_3
3PM. 5/2/58 Initial Reading	0, 10.7	10.7, 21.4	21.4, 32.1	32.1, 44.3 → 12.2 (12.1)	1.3, 13.4 → 12.1	13.4, 25.5	25.55, 35.25 → 9.70	35.25, ~~45.0~~ 44.95, 9.70	~~35~~ 41.1, 9.7
phenolph. ind.	10.7	10.7	10.7	12.1	12.1	12.1	9.7	9.7	9.7
3PM 7/2/58	0.2, 11.0 → 10.8	11.0, 21.8 → 10.8 (10.7)	21.8, 32.5 → 10.7	32.6, 44.7 → 12.1	0.3, 12.4	12.45, 24.6 → 12.15	24.6, 34.35 → 9.75	34.35, 44.15, 9.8	1.4, ~~9.20~~, 11.15, 9.75
	10.8	10.7	10.7	12.1	12.1	12.15	9.75	9.8	9.75
11.A.M. 10/2/58	0.15, 10.85 → 10.7	10.85, 21.65 → 10.80	21.7, 32.3 → 10.6	32.3, 44.5 → 12.2	0.3, 12.45	12.5, 24.55	24.5, 34.25	34.3, 44.1	18.5, 28.9
	10.7	10.8	10.6	12.2	12.15	12.05	9.75	9.8	~~10.4~~
11.15AM 12/2/58	0, 10.7	10.7, 21.5	21.5, 32.1	32.1, 44.3	0.1, 12.2	12.2, 24.3	24.35, 34.1	34.1, 43.9	19.3, 29
	10.7	10.8	10.6	12.2	12.1	12.1	9.75	9.8	9.7
17th	10.7	10.75	21.65, 32.1 → 10.65	32.2, 44.3 → 12.1	0.6, 12.7 → 12.1	12.8, 24.9 → 12.1	24.9, 34.6 → 9.7	34.7, 44.4 → 9.7	

24.77%

20.77%

430 / 207

.0414 − 0.0086

43 / 207

For finding out order of a reaction

Different concentrations. — Temp must be constant.

After Find out oxygen concentrn. at zero time.

after 24 hrs.
48 hrs.
3 days
4 days
5 days
6 days
~~7 days~~.

one for infinity.

Prepare 9 test tube cultures for each concentrn all of them being exactly similar Take 10 c.c in each test tube

2g/100 c.c 4g 8g. 10g. Seed with one drop.

Test cultures containing

paraformaldehyde 0.1%.

Sodium bisulphite 0.1%.

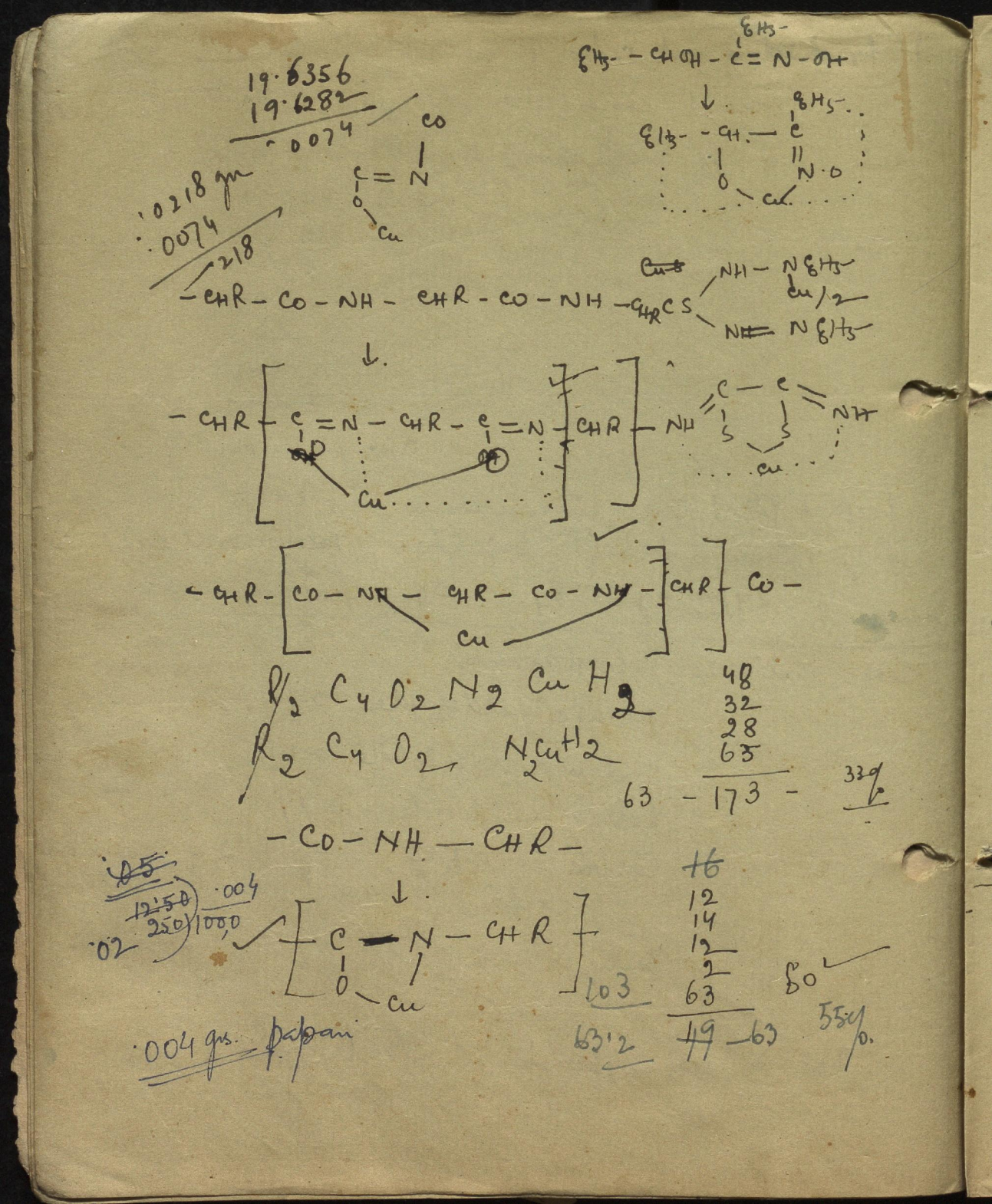
Ferric Chloride 0.1%

Carbon — 0.5 gm, 1g 2g. in total culture

control

Only with Al_2O_3.

Use in all experiments. B. Rajnyxa (4)


19·6356
19·6282
·0074
·0218 gm
·0074
218
CO
N
O
Cu
CH
N
O
Cu
NH – NC6H5
Cu/2
NH = NC6H5
CHR – CO – NH – CHR – CO – NH – CHR
CHR
NH
S
Cu
CO –
R2 C4 O2 N2 Cu H2
R2 C4 O2 N2 Cu H2
48
32
28
65
63 – 173 –
– CO – NH – CHR –
·02
·005
·004
12·50
250
100,0
103
·004 gms. papain
63·2
49 – 63
55%

I 20.77% 22.8294 .0086

Cu. .0262 Papain 1st Ferm 22.8708 .0414 22.8380 .0328 79.24 %

II 24.77% 32.11% 19.6278 62.98 %

Cu .0114 19.6282 II I III 19.6356 - 19.6352 .0144 52.29 %

Ferment 1st Ferm. 19.6500 .0218

Cu .0253 III 23.0698 23.0860 58.00

Fr. 2nd Papain 23.0704 23.0838 - 23.0838 .0318

23.1156 .0452

To 10 c.c. milk, 10 c.c. water, 2 c.c. Toluene Feb. P.M.

(1) Papain 1st Fraction by Equal 50% alcohol. 2-20 Completely clotted on 10/2/58

clotted 10/2/58

(2) Papain 2nd Fraction 80% alcohol — 2-22 C. clotted 12/2/58

(3) Papain with $CaCl_2$ — 2-24 C. clotted 12/2/58

clotted 10/2/58

(4) Fermented with alcohol + acetone — 2-26 C. clotted 12/2/58

clotted 10/2/58

(5) Ferm with $CaCl_2$ — 2-28 compl. clotted 12/2/58

(6) Papain — 2-30 wil clotted on 12/2/58

I 22.8276

Papain copper + Ca apex 22.8546 — 22.8430

II By 19.6257 .0116

Fermented. Cat F fraction 19.6610 gr .0160

19.6450

Effect of Zinc Sulphate.

(165)

Prepare 5 cultures

Dissolve 0.2 gm in 100 c.c

Add 0 c.c
5 c.c
12 c.c
15 c.c
20 c.c

150 c.c + 50 c.c seed culture.
200 c.c. Cultures

Due date for Analysis 26th. / 27th Feb or after 7 days.

The cultures contain.

K_2HPO_4 — 0.70
KH_2PO_4 — 0.30
$MgSO_4$ — 0.16
$(NH_4)_2SO_4$ — 0.34
Sugar. — 10g.

Zinc sulphate. — zero, 0.01, 0.02, 0.03, 0.04.

Org. used — Org. no: 4

18th

Date of seeding — 11/2/58. II Analysis 21/3/58

Analysis 27/2/58 16 days

Weigh the following — 14g.

3g { KH_2PO_4 — 6g
7g { K_2HPO_4 — 14g } Dissolve in 500 c.c 25 c.c will contain necessary phos.

1.6 { $MgSO_4$ — 3.2 g. } Dissolve in 500 c.c.
3.4 { $(NH_4)_2SO_4$ — 6.8 g. } in 25 c.c.

Dissolve in 1 litre. 50 c.c of this solution contains all the minerals. Weigh sugar separately.

Seed Culture Analysis.

T. Sugar: (Zinc) b. dist.

0
36.4 / 05.45

$\frac{10.9 \times 5}{100} =$ 0.5 — 100
— 10.9

I Analysis Date 27/12/58.

Acid in c.c. N/20 alkali.

1.	2.	3.	4.	5.	6.
0 → 0.2	0.2 → 0.35	0.35 → 0.55	0.55 → 0.75	0.75 → 1.0	1.0 → 1.15
0.2	0.15	0.2	0.2	0.25	0.15

7	8	9	10	11	12	13
1.30 → 1.5	1.15 → 1.3	1.5 → 1.7	1.7 → 1.9	1.9 → alk	alk	0.1
0.15	0.15	0.2	0.2	—	—	

Red sugar 20 → 50 c.c. New Fehling's soln.

1	2	3	4	5	6
10.6 → 15.0	13.0 → 16.6	15.7 → 19.7	11 → 15.5	9.0 → 14.3	13.6 → 18.0
4.4	3.6	4.0	3.5		

7	8	9	10	11	12	13
12.7 → 18.2	11.3 → 16.2	12.0 → 16.5	~~10.2~~ 10.2 → 14.9	13.5 → 23.4	9.5 → 16.3	11.8 → 17.6

5 c.c + 5 c.c of Fehling solution equal to 10.9 c.c of soln. (Sugar 0.5 g. in 100 c.c) 0.0545 g. sugar

Effect of carbon. III A - 14, 15, 16, 17.

Carbon — zero

0.5 g

1 g

2 g in total culture.

Prepare 4 normal cultures and (150 c.c. + 50 c.c. seed culture) (200 c.c.)
add the above.

Org used. — Rio/1: 4

Date of seeding — 11/2/58.

I Analysis — 20/2/58

II " — 13/3/58

Analysis of seed culture. Saccharose portions.

Total Sugar — (Carbon) B. diet. wrong Ri salt.

~~1.1~~
~~55.9~~
(58.1)

C_1	1.1100	1.1376	.0276
C_2	1.1060	1.1438	.0378
C_3	1.1256	1.1490	.0294
C_4	1.1220	1.1526	.0306

To be analysed on 27/2/58.

Analysed on 20/2/58.

Indicates very good growth.

Acid n/20 alk.	Red Sugar 25 → 10	T. Sugar inv. c	Butanediol nickel salt.
1 alk.	9.1	35.8 ~~38.0~~	~~II~~
2 alk	14.0.	42.2	~~III~~ I
3 alk	20.1 17.9 38.8 /20.9	26.6	III
4 0.65 n/20 alkali	17.1 1	25.8	nil.

Zinc.	Carbon	X	(2) I	(1) II	III	
1.1300	1.1300	1.1380	1.1680	1.1380	1.1500	Tri Salt

wts negligible in all the six cases.

I II III IV

Sugars

Effect of Aluminic. 6, 7, 8, 9

Prepare four normal cultures. (200 c.c)
Add — 0g, 0.5g, 1g. and 1.5g. in total.

Organism used — no: 4

Date of seeding — 18/2/58
analysis — 27/2/58 Reanalysis 21/3/58
Seed with 50 c.c of seed culture.

Seed culture Analysis (for antiseptic alk)
Total Sugar — B. diol.
wt of ri salt. X

1.6
29.5

Reanalysed Carbon Set on. 13/3/58

	I	II	III	IV
Red Sugar 25→50				
Acid 4.4 / 4.7	0.3	4.75 / 5.0 → 0.25	5.0 / 6.1 → 1.1	6.1 / 7.3 → 1.2
R.S. 25→50	11.6 / 16.7 → 4.9	12.4 / 18.5 → 6.1	16.9 / 27.1 → 10.2	13.2 / 22.4 → 8.2
Final Weight	0 → 34.5	0 → 33.5	0 → 36.1	0 → 35.0
Weight	·6534	·6700	·6486	·6642
	0.6900	·6976	·7038	·6742

I Analysis

II Analysis

I	1·1558 — 1·1754	·0196
II	1·1582 — 1·1796	·0214
III	1·1434 — 1·1646	·0212
IV	1·1256 — 1·1398	·0142
V	1·1536 — 1·1846	·0290
VI	1·1658 — 1·1768	0·0110
VII	1·1656 — 1·1896	·0240
VIII	1·1868 — 1·2016	0·0148
IX	1·1584 — 1·1446	
X	1·1264 — 1·1602	0·0338
XI	1·1394	nil.
XII	1·1464 — 1·1652	0·0188
XIII	1·1540 — 1·1652	·0112

Effect of different antiseptics. Prepare 4 cultures

① Normal culture (Total volume) – 200 c.c

Fluorescence observed.

② " + paraformaldehyde. 0.1 gm ~~0.01~~

③ " + Sodium ~~Sulphite~~ sulphite. 0.1 gm

④ " + Ferric ~~Chloride~~ sulphate. 0.1 gm.

Seeded with 50 c.c of org. no: 4

on date — 18/2/58.

Analysis date — 27/2/58 9 days [10, 11, 12, 13]

Date of reanalysis — ~~17/3/58~~ 21/3/58

II Analysis:

	1	2	3	4	5	6	7
Red	8.1 → 8.4	8.4 → 8.8	8.8 → 9.1	9.1 → 9.3	9.3 → 9.5	9.5 → 9.85	9.85 → 10.15
	0.3	0.4	0.3	0.2	0.2	0.35	0.30

	9	9	10	11	12	13.
	10.15 → 10.4	10.6 → 10.7	10.7 → 11.0	c/2	11.0 → 11.30	11.35 → 11.6
Red	0.25	0.3	0.3	–	0.3	0.35

	I	II	III	IV	V	VI	VII	VIII	IX
25 → 50	11.5 → 17.0	13.0 → 20.4	10.5 → 16.9	22.1 → 27.7	11.0 → 18.5	9.2 → 14.3	10.8 → 15.8	~~15.8~~ 11.2 → 16.7	14.9 → 19.4
	5.5	7.4	6.4	5.6	7.5	5.1	5.0	5.7	4.5

	X	XI	XII	XIII
	11.7 → 17.1	11.9 → 19.1	~~13.4~~ → 18.7	10.5 → 15.0
	5.4	7.2	5.3	4.5

Total sugar

I Analysis

1 – 38.8
2 – 38.0
3 – 44.0
4 – 43.8
5 – 43.0
VI – 54.2 ✓
7 – 38.0
8 – 43.2
9 – 41.8
10 – 50.7 ✓
11 – 45.2
12 – 38.3
13 – 47.2

I – 22.8274
varieis 22.8590 0.0316
ash – 22.8310 0.0036 (11.4 %)

II – 19.6254
– 19.6546 – 0.0292
19.6320 0.0066
(22.6 %)

.0316 – .0036

10 – $\frac{100 \times .0036}{.0316} = \frac{360}{79}$ 11.4 %

22.6

.292 – .066

10 – $\frac{100 \times 66}{292} = \frac{33}{14.6}$

Total sugar II Analysis

I	II	III
45.3	36.8	38.0

IV 39.5 V 40.9 VI 40.6 VII 39.4 VIII 38.5

IX 35.7 X 32.0 XI 61.5 XII 37.3

XIII 43.3

11/2/58

	I	(2) II	2·5 III	2·15 IV	2·40 V	3·20 VI
burnt K	0	1·9	3·9	7·7	5·8	9·65
2	1·9 > 1·9	3·8 > 1·9	5·7 > 1·8	9·65 > 1·95	7·7 > 1·9	11·55 > 1·90
+S.C.C formal	2·9 > nil	nil	nil		nil	
	I 1·9	1·9	1·8	1·95	1·9	1·90.

~~2·47~~

		2·55	3·00	3·05	3·10	3·20
2·47.	11·7 >	13·55 >	15·4 >	17·3 >	19·2 >	21·0 > 1·9
	13·5	15·40	17·2	19·1	21·0	22·9
	II 1·8	1·85	1·8	~~1·8~~ 1·8	1·8	1·9

12·20 ~~pm~~ am	III 1·8	1·85	1·85	1·9	1·85	1·9

15c 8 m. +formal. boiled.

Regd alkali	2 drops	4 drops	2 drops	3 drops	4 drops.	2 drops.

12·45 on 10/2/58.	0·25	2·1	4·3	6·4	8·9 >	10·9 > 1·9
	2·1 > 1·85	4·0 > 1·9	6·2 > 1·9	8·85 ~~8·45~~ >	10·7	12·8
				excess 2·45	1·8	
	+ formal. 2 drops	+ formal 3 drops	formal 4 drops	+ formal: + final color became v. faint	4 drops	3 drops

no ppt in (II)

2h 2·42 in hot water.

I — 3·10

(1)	(2)	(3)	(4)	(5)	(6)	(7)
·6460	·6244	·6256	·6644	·6598	·6826	·6638
~~·8~~ ·6684	·6425	·6580	6880	·6944	·7276	·6838

(8)	(9)	(10)	(12)	(13)
·6094	0.6366	0.6348	1·1394	0·6250
·6350	·6568	0·6588	1·1594	·6460

$\frac{13.5 \times N}{20} = \frac{19.95 \times N}{20}$

$x = \frac{27 \cdot 675 \cdot}{13.5} = \frac{20 \times 19.95}{4}$

675) 19150 (28.
1350
5450
5400
500

Cryoanthemum

40) 53 (1.325
40
130
120
100
80
200
200

$\frac{N}{28}$.

0.53 - N
$\frac{53}{2}$ - N
$\frac{N}{20}$

70
295
50
445

$\frac{53}{2 \times 20}$

Calculations.

31.3168
5

31.3168 (20
1.325 (32
32.641

59 4
5
295

4

Calculations.

Milk Clotting.

I — 2.20
II — 2.22
III — 2.24
IV — 2.26
V — 2.28
VI — 2.30.

Analyses. after 5/2/58 done with alk of following strength.

10 c.c Exact N/20 Na_2CO_3 requires 13.5 ~~4.6~~ c.c of HCl.

10 c.c. The alkali in question requires 19.95 c.c of same HCl

Rangam

HB pencil

Rangam

Rangam Rangam

(1)

(2)

(3)

(4)

(1) SM in Sugar — A 3%. B 6%. C 12%.

(1) Starch — D 3%.

B.S. in Starch — 6%. E

(2) SM in Sugar 3% Milk. 1 1% 2 2% & 4%

(2) Starch 3% Milk 1% 2% 3% & 4%
4 5 6 7

(3) SM in Sugar 3% } — phosphate. 4 5cc, 5 10cc & 6 15cc
milk 2% } soln.

I (3) Starch 3% } " 5cc 10cc & 15cc.
milk 2% } 1 2 3

(4) B.S in sugar 3% with $CaCO_3$ 2g

Starch 3% with $CaCO_3$ 2g

(4) S.M in sugar 3% with " milk 2cc

Starch 3% with "

Investigating the influence of sucrose concentration on the formation 2,3. butanediol in Serratia marcescens and ~~observing~~ effect of neutralisation of the acids formed in the culture by $CaCO_3$ on the yield of diol.

I

Acid Titre value.

Strength of Na_2CO_3 = ~~0.236 N~~ = 0.01 N

					gm equiv of acid
I.	A.	nil			—
50 c.c of soln.	B.	2 c.c.	~~0.118 × 2~~	=	~~0.059~~. 0.0094
	C.	3.4 c.c	~~0.118~~/3.4	=	~~0.035~~ 0.0006
	D.	no acid.	—		—
	E.	1.3.		=	0.09.

III

II Red sugar. 5 c.c diluted to 50 c.c

A nil

B —

C —

D nil

E nil

Total sugar. 5 c.c hyd ⇀ → 50 c.c. gm of sugar (invert)

Titrated with 5+5 c.c Fehling's.

4 → 50 c.c A · 25.5. — $\frac{50 \times 0.05 \times 100}{25.5 \times 4}$ 2.469 gm

B 5 c.c — $\frac{50 \times 0.05 \times 100}{5 \times 5}$ 5.8. 10 gm.

C 3.8 c.c — $\frac{50 \times 0.05 \times 100}{3.8 \times 5}$ 13.16 g.

D. 20.1 c.c. — 50/20.1 2.49 11.5

E. 18.4 c.c — 50/18.4

Phenyl hydrazone. 5 c.c in 100 c.c.

A — negligible. 0.008. 0.008 × 0.202 × 20 = 0.03232

B — 0.02 g. 0.02 × 0.202 × 20 = 1.0808

C — 0.01 g. 0.01 × 0.202 × 20 = 0.0404

D — 0.007. 0.007 × 0.202 × 20 = 0.02828

E — 0.055 0.055 × 0.202 × 20 = 0.2222

$\frac{4.04 \times 0.02}{.0808}$

② Acid

1 alk

2 alk

3 5.7 ~~0.118/5.9~~ = ~~0.02~~ 0.0011

4 alk

5 alk

6 1.5 ~~0.118/1.5~~ = = ~~0.079~~ = 0.0003

7 alk.

Red. sugar. no dilution.

1 — 31.8 - 0.05
100 : $\frac{100 \times 0.05}{31.8}$

2 —

3 31.8 c.c. 5/31.8 = 0.16

4 → 40 c.c. 5/40 = ~~0.20~~ 0.125

5 → 50 c.c. 5/50 = 0.10

6 → 20.1 c.c. 5/20.1 = 0.25

7 → 6.8 c.c 5/6.8 = 0.74

Total sugar.

5 c.c ⇒ 50 c.c

1	15.8	$\frac{450 \times 0.05 \times 100}{15.8 \times 5}$	= 3.17
2	16.4	50/16.4	= 3.05
3	22.6	50/22.6	= 2.21
4	18.0	50/18	= 2.78
5	22.5	50/22.5	= 2.22
6	26.1	50/26.1	= 1.92
7	26.3	50/26.3	= 1.90

Phenyl hydrazone.

Butylene glycol

1 —	0.0240	0.024 × 0.202 × 20 = 0.097
2 —	0.0348	0.035 × 0.202 × 20 = 0.141
3 —	0.05	0.05 × 0.202 × 20 = 0.202
4 —	0.0200	0.02 × 0.202 × 20 = 0.081
5 —	0.0302	0.03 × 0.202 × 20 = 0.122
6 —	0.0208	0.021 × 0.202 × 20 = 0.085
7 —	0.0250	0.0250 × 0.202 × 20 = 0.101

3/ Acid

1 alk

2 alk

3 1.7 ~~0.118/1.7~~ = ~~0.066~~ 0.0003

4 1.3 ~~0.118/1.3~~ = ~~0.091~~ = 0.0003

5 6.3 ~~0.118/6.3~~ = ~~0.018~~ = 0.0013

6 alk.

Red Sugar: No dilution

1 & 2 —

3. 30.8 5/30.8 — 0.16

4. 3.8 5/3.8 — 1.32

5 3.2 5/3.2 1.56

6. 13.9. $5/13.9 = 0.36$

Sugar after hydrolysis $5 \rightarrow 50$.

① 24.7 — $50/24.7 = 2.02$

② 24.8 $50/24.8 = 2.02$

③ 26.5 $50/26.5 = 1.89$

3.7 ④ 12.7 $50/12.7 = 3.94$

2.88 ⑤ 17.7 $50/17.7 = 2.82$

⑥ 37.7 $50/37.7 = 1.33$

Phenyl hydrazone:

1 — 0.021 × 4.04 = 0.085

2 — 0.012 × 4.04 = 0.048

3 — 0.004 × 4.04 = 0.016

④ — 0.044 × 4.04 = 0.178

5 — 0.099 × 4.04 = 0.38

6 — 0.063 × 4.04 = 0.255

---

40 → 100
84

$\frac{250}{20}$ 12.5

84 — 0.05

100 → $\frac{100 \times 0.05}{84}$ — 40

$\frac{250 \times 100 \times 0.05}{60 \times 84}$ — $\frac{100}{12.5}$ / 84

(4) I Acid Sugar. 20 → 50.

18.3. $\frac{18.3 - 0.05}{50}$ $\frac{50 \times 0.05}{18.3} \times \frac{100}{20} = \frac{12.5}{18.3} = 0.68$

II 42.4 $\frac{12.5}{42.4} = 0.29$

III & IV — negligible.

Total Sugar:

I — 9.7 12.5/9.7 = 1.3

II — 22.4 12.5/22.4 = 0.56

III & IV — absent

Phenyl hydrazone. I — 0.014 × 4.04 = 0.057

II — 0.024 × 4.04 = 0.097

III — 0.009 × 4.04 = 0.036

IV — —

Comparative Study.

Acid = 10 c.c.

1	alk		
2	2.2	~~0.118/2.2~~	0.0002
3	alk		
4	alk		
5	2.8	~~0.118/2.8~~	0.0003
6	2.6	~~0.118/2.6~~	0.0003
7	3.2	~~0.118/3.2~~	0.0003
8	1.5	~~0.118/1.5~~	0.0001
9	2.0	~~0.118/2.0~~	0.0002
10	2.4	~~0.118/2.4~~	0.0002
11	alk		
12	alk		

Reducing sugar :- 20 → 50 c.c

1	—		
2	84	12.5/84	0.1488
3	—		
4	84	12.5/84	0.1488
5	6.5	12.5/6.5	1.91
6	86	12.5/86	0.1453
7	24.2	12.5/6.5	1.91
8	80	12.5/86	0.1453
9	7.0	12.5/7.0	1.785
10	6.6	12.5/6.6	1.894
11	—		
12	—		

Total Sugar 20 → 50cc

1	4.9	12.5/4.9	= 2.55
2	4.2	12.5/4.2	= 2.98
3	5.2	12.5/5.2	= 2.41
4	5.0	12.5/5.0	= 2.5
5	6.1	12.5/6.1	= 2.05
6	5.0	12.5/5.0	= 2.5
7	5.0	12.5/5.0	= 2.5
8	5.5	12.5/5.5	= 2.27
9	6.2	12.5/6.2	= 2.02
10	6.0	12.5/6.0	= 2.08
11	4.4	12.5/4.4	= 2.84
12	4.5	12.5/4.5	= 2.78

Phenyl hydrazone in 5 c.c

1	0.04	0.04 × 0.202 × 20	0.1616
2	0.028	0.028 × 4.04	0.1132
3	0.04	0.04 × 4.04	0.1616
4	0.025	0.025 × 4.04	0.101
✓5	0.07	0.09 × 4.04	0.2828
✗6	0.01	0.01 × 4.04	0.0404
✓7	0.055	0.055 × 4.04	~~0.2~~ 0.222
✗8	0.047	0.047 × 4.04	0.1899
9	0.072	0.072 × 4.04	0.2909
✓10	0.073	0.073 × 4.04	0.2949
✗11	0.027	0.027 × 4.04	0.7091
12	0.037	0.037 × 4.04	0.1495

$ZnSO_4$. Cultures. Strength of acid $\frac{1}{11.8 \times 2}$

	gg Calc. left.	5c.c.	
1	0.2	$\frac{1}{128}$	$\frac{1}{118}$
3	0.2	$\frac{1}{128}$	$\frac{1}{118}$
4	1.1	$\frac{11}{236}$	
5	0.4	$\frac{2}{118}$	
6	0.3	$\frac{3}{236}$	

2 (Thr. with alk) 1.2 2.4 0.0002

Red Sugar 20c.c → 50c.c.

1	17.0	12.5/17	0.7224
2	15.7	12.5/15.7	0.6324

3 43.2 12.5/43.2 0.2894

4, 5 & 6 — no redn.

Total sugar 20 → 50 c.c

① 8.1 12.5/8.1 1.543

② 7.6 12.5/7.6 1.644

③ 14.3 12.6/14.3 0.8742

4, 5 & 6 — nil

Phenyl hydrazone

1) 0.0715 4.04 × 0.0715 0.2889

② 0.0900 4.04 × 0.09 0.3636

③ 0.0558 4.04 × 0.0558 0.2254

4 0.005 $4.04 \times 0.005 = 0.0202$

5 0.006 $4.04 \times 0.006 = 0.024$

6 0.001 $4.04 \times 0.001 = 0.004$

Bacillus Subtilis + phosphate.
Sugar Culture:—

Acid formed. titre with Na_2CO_3. (in 10 c.c)

1. 2.5 $\frac{25 \times 0.01}{1000} = 0.00025$

2. 3.0 $\frac{30 \times 0.01}{1000} = 0.0003$

3. alkaline.

Comparitive study with starch

Red sugar. ~~titr with dichromate~~. 20c.c → 50c.c

1. 10.5 12.5 | 10.5 1.191

2. 10.8 12.5 | 10.8 1.157

3. 45c.c 12.5 | 45 0.2777

Total sugar. 20c.c → 50c.c.

1. 11.2 12.5 | 11.2 1.116

2. ~~29.5~~ 7.2 12.5 | 7.2 1.736

3. 29.5 12.5 | 29.5 0.4239

Phenyl hydrazone

1. 0.119	4.04 × 0.119	0.4807
2. 0.12	4.04 × 0.12	0.4849
3. 0.096	4.04 × 0.096	0.3879

Starch Cultmer.

Acid.

1) 0.9	$\frac{9 \times 0.01}{1000}$	0.0001
2) 2.8	$\frac{28 \times 0.01}{1000}$	0.00028
(3) 2.1	$\frac{21 \times 0.01}{1000}$	0.00021

Red Sugar. titr. with dichromate. (in 10 c.c)

10 c.c : ~~0.0315 gm~~ 0.0028 g of sugar

1. 10.2	10 × 10.2 × 0.0028 =	0.2859
2. ~~10.8~~ 11.8	11.8 × 10 × 0.0028	0.3305
3). 7.6	7.6 × 10 × 0.0028	0.2128

Total Sugar in 10 c.c.

1.	11.5	11.5 × 10 × 0.0028	0.3223
2.	18.3 (10.4)	18.3 × 10 × 0.0028	0.5125
3.	9.3. (8.3)	9.3 × 10 × 0.0028	0.2604

Phenyl hydrazone:

1.	0.0130	4.04 × 0.013	0.0525
2.	0.0140	4.04 × 0.014	0.0566
3.	0.005	4.04 × 0.005	0.0202

Comparative study with starch:

Acid formed.

1	4.9	$= \frac{49 \times 0.01}{1000}$	= .00049
2	8.7		= .00087
3	4.1		= .00041
4	alk		—
5	6.4		0.00064
6	alk		—
7	4.8		0.00048
8	1.1		0.00011
9	11.2		0.00112
10	4.5		0.00045
11	alk		—
12	5.9		0.00059

Red Sugar in 10 c.c with dichromate.

1	8.4	$8.4 \times 10 \times 0.0028 =$	0.2353
2	7.0	$7.0 \times 10 \times 0.0028$	0.196
3	8.1	8.1×0.028	0.2269
4	27.5	27.5×0.028	0.7700
5	12.2	12.2×0.028	0.3416
6	7.0		0.1960
7	10.8		0.3024
8	20.1		0.5628
9	26.0		0.7281
10	8.7		0.2436
11	3.1		0.0868
12	2.4		0.0672

Total sugar in 10 c.c.

1	18.1	18.1 × 0.028	= 0.5069
2	16.8		= 0.4704
3	20.1		= 0.5628
4	43.0		= 1.204
5	21.0		= 0.588
6	29.2		= 0.8177
7	19.1		= 0.5348
8	28.2		= 0.7896
9	28.4		= 0.7952
10	18.8		= 0.5265
11	10.2		= 0.2856
12	9.6		= 0.2688

Phenyl hydrazone:

1	0.001	4.04 X	0.001	= 0.00404
2	0.001			= 0.00404
3	0.001			= 0.00404
4	0.0840			= ~~0.1156~~ 0.3394
5	0.0780			0.3159
6	0.0360			0.1454
7	0.001			0.00404
8	"			"
9	"			"
10	"			"
11	"			"
12	"			"

Effect of concentration on B. Subtilis. Sugar

Acid in 5c.c			
1	1.8	3.6 →	0.00036
2	3.0	6 →	0.0006
3	2.6	5.2 →	0.00052
4	2.5	5 →	0.0005

Red Sugar in 5c.c.

1.	13.2	13.2 × 20 × 0.0028	0.7392
2.	26.1	26.1 × 20 × 0.0028	1.462
3.	28.0	28.0 × 20 × 0.0028	1.568
4.	35.7	35.7 × 20 × 0.0028	1.999

Total sugar in s.c.c

1.	13.4	13.4 × 20 × 0.0028	0.7504
2.			—
3.	70.3	70.3 × 20 × 0.0028	3.938
4.	146.9	146.9 × 20 × 0.0028	8.226

Phenyl hydrazone:

1).	0.096	4.04 × 0.096 = 0.3879
2)	0.093	4.04 × 0.093 = 0.3758
3)	0.094	4.04 × 0.094 = 0.3798
4)	0.082	4.04 × 0.082 = 0.3313

Starch. Acid in 5c.c

1. 1.5 $1.5 \times \frac{20 \times 0.028}{10} \times$ = 0.0003

2. 1.7 $1.7 \times \frac{20 \times 0.028}{34}$ 0.00034

3. 1.9 $1.9 \times 20 \times 0.028$ 0.00038

4. 1.4 $1.4 \times 20 \times 0.028$ 0.00028

Red Sugar in 5c.c

1. 4.4 $4.4 \times 20 \times 0.0028$ = 0.2464

2. 11.3 $11.3 \times 20 \times 0.0028$ = 0.6328

3. 9.6 $9.6 \times 20 \times 0.028$ = 0.5376

4. 4.1 $4.1 \times 20 \times 0.028$ = 0.2296

Total sugar in 5 c.c.

1.	6.1	6.1 × ~~4.04~~ 20 × 0.0028	0.3416
2.	20.4	~~6.1~~ 20.4 × ~~4.04~~ "	1.142
3.	30.1	30.1 × ~~4.04~~ "	1.686
4.	21.1	21.1 × ~~4.04~~ "	1.1181

Phenyl hydrazone:—

0.001 × 4.04 = 0.00404

—— × ——

Effect of $CaCO_3$ on SM

II

Acid formed.. calc. by titrating $CaCO_3$ left. 10c.c culture

1) 11.4

2) 31.3

3) 8.0

4) 4.1

5) 6.4

6) 3.2

Reducing sugar. 5c.c.

1)	22.1	22.1 × 20 × 0.0028	1.237
2)	11.2	11.2 × 20 × 0.0028	0.6272
3)	13.7	13.7 × 20 × 0.0028	0.7672
4)	17.6	17.6 × 20 × 0.0028	0.9856
5)	3.4	3.4 × 20 × 0.0028	0.1904
6)	16.6	16.6 × 20 × 0.0028	0.9296

Total Sugar in 5 c.c.

1) 25.6 — 25.6 × 20 × 0.0028. = 1.433

2) 21.3 — 21.3 × = 1.193

3) 16.3 — 16.3 × = 0.9128

4) 26.4 — 26.4 × = 1.479

5) 7.4 — 7.4 × = 0.405

6) 15.5 — 15.5 × = 0.8682

Phenyl hydrazone:

1) 0.106 — 0.106 × 4.04 = 0.4283

2) 0.093 — 0.3758

3) 0.099 — 0.3999

4) 0.073 — 0.2948

5) 0.044 — 0.1778

6) 0.091 — 0.3676

Effect of Phos on S.M. All are alkaline I

Std.	Red sugar in 10 c.c	
I	4.3	4.3 × 0.028 = 0.1204
II	3.7	3.7 × 0.028 = 0.1036
III	4.6	4.6 × 0.028 = 0.1288
1	2.0	2.0 × 0.028 = 0.05616
2	1.4	1.4 × 0.028 = 0.03920
3	2.7	2.7 × 0.028 = 0.0756
X	6.7	6.7 × 0.028 = 0.1876

Total Sugar — in 10 c.c.

I	47.9	47.9 × 0.028	1.342
II	64.1	64.1 × 0.028	1.795
III	91.1	91.1 × 0.028	2.551
1	44.8	44.8 × 0.028	1.254
2	50.2	50.2 × 0.028	1.406
3	46.0	46.0 × 0.028	1.288
X	51.4	51.4 × 0.028	1.44

Phenyl hydrazone

I	0.016	0.016 × 4.04	0.06465
II	0.015	0.015 × 4.04	0.0606
III	0.017	0.017 × 4.04	0.06868
1	0.02	0.02 × 4.04	0.08079 ~~0.04860~~
2	0.012	0.012 × 4.04	~~0.09696~~ 0.04860
3	0.024	0.024 × 4.04	0.09696
X	0.012	0.012 × 4.04	0.0486

Comp. study.

			Acid in 10 c.c	
1	4.9	3.2	3.2 × 0.0001	0.0003
2	8.7	4.2	4.2 × 0.0001	0.0004
3	4.1	2.1	2.1 × 0.0001	0.0002
4	alk	alk	—	—
5	6.4	2.7	2.7 × 0.0001	0.0003
6	alk	—		—
7	4.8	3.9		0.0004
8	4.1	2.7		0.0003
9	11.2	alk		—
10	4.5	4.6		0.0005
11	alk	3.1		0.0003
12	5.9	2.2		0.0002

Red sugar in 10c.c

1	8.4	15.5	15.5 × 0.028	= 0.434
2	7.0	8.1	8.1 × 0.028	= 0.2269
3	8.1	7.0	7.0 × 0.028	= 0.196
4	27.5	3.4	3.4 × 0.028	= 0.953
5	12.2	43.2	43.2 × 0.028	= 1.21
6	7.0	—	—	
7	10.8	32.9	32.9 × 0.028	= .92
8	20.1	0.9	0.9 × 0.0028 × 20	= 0.50
9	26.0	4.1	4.1 × 0.0028 × 20	= 0.23
10	8.7	27.5	27.5 × 0.028 × 2	= 1.54
11	3.1	4.5	4.5 × 0.028 × 2	= 0.2520
12	2.4	3.2	3.2 × 0.028 × 2	= 0.18

5 c.c

5c.c

Red Sugar

Total sugar in 10c.c

1	18.1	57.3	57.3 × 0.028 =	1.604
2	16.8	41.8	41.8 × 0.028	1.17
3	20.1	50.8	50.8 × 0.028	1.42
4	18.0	42.0	42.0 × 0.028	1.18
5	21.0	55.9	55.9 × 0.028	1.57
6	29.2	—	—	—
7	51.5		51.5 × 0.028	1.44
8	40.2		40.2 × 0.028 × 2	~~1.13~~ 2.25
9	84.3		84.3 × 0.028 × 2	~~2.36~~ 4.72
10	46.1		46.1 × 0.028 × 2	~~2.58~~ (1.29) 4.73
11	84.8		84.8 × 0.028 × 2	~~2.37~~ 3.18
12	56.8		56.8 × 0.028 × 2	~~1.59~~

5.c. Recalculate

~~Propyl~~ Butylene glycol in 5 c.c

1)	0.0650	0.065 × 4.04	~~.2626~~	0.26
2)	0.0180	0.018 × 4.04	–	0.073
3)	0.0600	0.0600 × 4.04		0.24
4)	0.023	0.023 × 4.04		0.093
5)	0.025	0.025 ×		0.101
7)	0.1070	0.107 ×		0.43
8)	0.063	0.063 ×		0.25
9)	0.005	× 4.04		0.02
10)	0.092	× 4.04		0.37
11)	0.003	× 4.04		0.012
12)	0.033	× 4.04		0.13

Red Sugar

Effect of Milk Acid in S.C. IV

1) 2.1 — 2.1 × 2 × 0.0001 — 0.0004

2) 3.2 — 3.2 × 2 × 0.0001 — 0.0006

— 0.0005

3) 2.7

— 0.0008

4) 2.9

— 0.0006

5) 3.0

— 0.0007

6) 3.7

Reducing sugar in 5c.c

1) 3.9 — 3.9 x 0.0028 x 20. — 0.2184

2) 7.0 — 0.392

3) 13.1 — 0.7336

4) 16.6 — 0.9296

5) 17.5 — 0.98

6) 5.5. — 0.308

Total sugar in 5c.c

1) 56.4 — 56.4 x 20 x 0.0028 — 3.975

2) 73.0 — 4.088

3) 43.7 2.447

4) 45.8 3.229

5) 40.5 2.269

6) 48.1 2.694

Phenyl hydrazone:

1) 0.0420 0.042 × 4.04 0.1698 0.17

2) 0.0560 0.056 × 4.04 0.2262 0.23

3) 0.0745 0.075 × 4.04 0.301 0.30

4) 0.0930 0.093 × 4.04 0.3758 0.38

5) 0.0926 0.093 × 4.04 0.3741 0.37

6) 0.0690 0.069 × 4.04 0.2787 0.28

Sugar milk. Comparative study.

V

Acid in 10c.c

1	2.3	×0.0001	= 0.0002
			0.0091
2	11.6		
			0.0005
3	5.5		
4	1.6		
5	5.9		
6	4.0		
7	6.2		
8	alk		
9	22.4		
10	5.4		
11	2.2		
12	9.5		

Red Sugar in 5c.c

1	4.1	× 0.0028 × 2. = 2246	0.23
2	8.2		0.46
3	6.1		0.34
4	4.9		0.27
5	8.4		0.47
6	4.7		0.26
7	30		1.68
			0.57
8	10.1		0.35
9	6.2		0.41
10	7.3		0.27
11	4.9		0.21
12	3.8		

Total sugar in 5c.c

1 4.3 × 0.0028 × 2 = = 0.24

2 97.8 × 0.0028 × 2 : = 5.48

3 6.6 × 0.0028 × 2 : 0.37

4 14.4 × 0.0028 × 2 : 0.81

5 19.4 × 0.0028 × 2 Or 1.09

6 20.2 × 0.0028 × 2 = 1.13

7 48.6 2.72

8 54.8 3.07

9 13.4 0.75

10 23.9 1.34

11 43.6 2.44

12 8.4 0.47

Red Sugar

Butylene glycol

1	0.023 × 4.04 =	0.093
2	0.014	0.057
3	0.038	~~[illegible]~~ 0.154
4	0.014	0.057
5	—	—
6	0.009	0.036
7	0.038	0.154
8	0.009	~~0.234~~ 0.036
9	0.008	0.032
10	0.029	0.117
11	0.023	0.093
12	0.019	0.077

Red Sugar

Butterscotch

1	0.023	X4.04 =	0.093
2	0.014		0.057
3	0.038		0.154
4	0.016		
5	—		
6	0.009		
7	0.038		
8	0.009		0.036
9	0.008		0.032
10	0.029		0.117
11	0.023		0.093
12	0.019		0.077

2.48
201) 500
402
980
804
1768
1608
1520
2.71
184) 500
368
1320
1288
320
184
136

Red Sugar

Butylene glycol

1	0.023	X4.04	=	0.093
2	0.014			0.057
3	0.038			0.154
4	0.01[illegible]			0.057
5	—			—
6	0.009			[illegible]
7	0.03[illegible]			
8	0.00[illegible]			0.036
9	0.008			0.032
10	0.029			0.117
11	0.023			0.093
12	0.019			0.077

Ward, Pettijohn, Lockwood, + Coghill, J. Am. Chem. Soc., 86, 541, (1947)

Molasses

Butane diol.

Acid insc. cv.

1) 18.4 — 0.0037

2) 11.7 — 0.0023

3) 5.5 — 0.001

4) 6.0 — 0.0012

5) 15.2 — 0.003

6) 19.3 — 0.0039

7) 20.2 — 0.004

8) 19.8 — 0.004

9) 7.6 — 0.0015

10) 19.6 — 0.0039

11) 13.1 — 0.0026

12) alk —

13) 13.7 0.0027

14) 10.4 0.002

15) 8.1 0.0016

Red Sugar ∴ S.C.

1) 78 × 0.0028 × 20 = 4.368
2) 59.3 × 0.0028 × 20 = 3.321
3) 48.3 × = 2.705
4) 35.8 = 2.004
5) 32.7 = 1.831
6) 30.3 = 1.696
7) 24.0 = 1.344
8) 16.6 = 0.9301
9) 35.4 = 1.983
10) 21.8 = 1.537
11) 42.4 = 2.374
12) 6.2 = 0.3472
13) 23.8 = ~~1.332~~ 1.332
14) 15.5 = 0.868
15) 22.5 = 1.26

Total Sugar

1) 181.8 — 10.2

2) 124.6 — 6.98

3) 135 — 7.56

4) 10.3 — 0.58

5) 40.7 — 2.28

6) 125.8 — 7.051

~~7) 10.2~~

7) 169 — 9.464

8) 166.9 — 9.353

9) 91.5 — ~~5~~.124 5.124

10) 46.7 — 2.615

11) 35.6 — 1.994

12) 14.0 — 0.784

13) 47.9 — 2.682

14) 43.0 — 2.408

15) 27.5 — 1.54

Red Sugar

Phenyl hydrazone

			% on the basis of Sugar consumed
1)	0.045		
2)	0.0145	— 0.059	1.89
3)	0.011	— 0.044	1.80
4)	0.0270	— ~~0.11~~ 0.11	~~0.11~~
5)	0.0340	— ~~0.14~~ 0.14	~~0.18~~
6)	0.0330	— ~~0.13~~ 0.13	~~3.97~~
7)	0.0280	0.028 × 4.04 — 0.1132	
8)	0.0390	× 4.04 = — ~~0.1576~~	
9)	0.0370	× 4.04 = — 0.1495	
10)	0.002	× 4.04 = — ~~0.008~~	
11)	0.002	— 0.008	
12)	0.0300	— 0.1212	
13)	0.0490	— 0.1980	
14)	0.0526	— 0.2125	
15)	0.0426	— 0.1720	

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Acid in 5c.c. Na_2CO_3 $N/20$. $Rq \times \frac{1}{500}$ Sugar

1) 0.6 — 0.0012

2) 1.0 — 0.002

3) 2.1 — 0.004

4) 2.1 — 0.004

5) 1.1 — 0.002

6) 0.5 — 0.001

7) 0.8 — 0.0016

Red Sugar in 5c.c.

1) 98.3 — 5.505

2) 86.4 — 4.838

3) 95.0 — 5.319

4) 99.0 — 5.544

5) ~~66~~.4 — 3.718 — Control.

6) 44.0 — 2.464 — Seeded with culture alk. T. Sugar - 0.1414 B. dist - 0.0148

7) 43.8 — 2.452 in ~~25~~ 50 c.c.

50 c.c. of Seeded milk + culture
Contr. no acid. in 50 c.c
R.S — ~~0.6664/4~~ = 0.1666
T.S — ~~+.691~~ = 0.423
B. dist. — 0.0275

Red Sugar

Sugar = 5 c.c

1) 15.6	— 0.8736	25	1.25
2) 27.1	— 1.518	50	2.5
3) 68.2	— 3.811	75	3.75
4) 41.5	— 2.324	100	5.0
5) 44.6	— 2.498	2.5 — 50	
6) 3.6	— 0.2016	0.4	
7) 14.4	— 0.8065		

15.6 × .112 / 312 / 156 / 156 / 1747 (2)

3.75 / 425 (2) / 4.173 / 3.81 / .363 — 8736

Phenyl hydrazone :-

1) 0.0154	— 0.062
2) 0.0240	— 0.097
3) 0.0335	— 0.135
4) 0.0428	— 0.173
5) 0.0358	— 0.145
6) 0.0300	— 0.121
7) 0.0142	— 0.057

Rq × 2 / 1000 — 1 / 1000 — .0002

.001

.1 = 1/10

.01 = 1/100

.001 = 1/1000

Acid in 5c.c.

1 – 3.9 0.0078

2 – 1.8 0.0036

3 – 1.7 0.0034

4 – 1.7 0.0034

5 – 1.2 0.0024

6 – 1.4 0.0028

7 – 1.3 0.0026

8 – 0.4 0.0008

Seed } in
culture } 50. —

Reducing Sugars in 5c.c
Total

1 – 1.8 – 0.2016

2 – 8.8 – 0.9856

3 – 8.9 – 0.9968

4 – 21.0 – 2.352

5 – 22.0 – 2.463

6 – 24.0 – 2.751

7 3.8 – 0.4256

8 20.6 – 2.330

0.54

Sucrose 19.2 – 2.15/4

Butane diol in

0.043 – 0.1738

0.0476 – 0.1923

0.0470 – 0.1899

0.0748 – 0.3022

0.0628 – 0.2537

0.0670 – 0.2707

0.0525 – 0.2121

0.0362 – 0.1462

0.0244 – 0.0986
4

[redacted]

Sugars in 5c.c
Red

19.7 – 2.207

22.0 – 2.463

33.7 – 3.774

15.6 – 1.747

15.9 – 1.78

17.3 – 1.937

16.6 – 1.86

39.4 – 4.413

0.31

11.0 1.232
4

Acid

1 — 0.8

2 — 11.8

Rest alk.

0.8 = 0.0016

11.8 = 0.0236

R. Sugar.

I	2.8 →	$2.8 = 0.0046$ $200 = \frac{200 \times 0.0046}{2.8}$	— 0.3286	3.29
II	1.2	$\frac{200 \times 0.0046}{1.2}$	— 0.7667	7.67
III	2.3	$\frac{200 \times 0.0046}{2.3}$	— 0.4	4.00
IV	7.4	$7.4 \times 0.0028 \times 20 \times 2$	— 0.8287	~~8.29~~ .828
V	13.1	$\frac{200 \times 0.0046 \times 4}{13.1}$	— 0.2809	2.81
VI	18.5	$\frac{200 \times 0.0046 \times 4}{18.5}$	— 0.1989	1.98
VII	11.5	$\frac{200 \times 0.0046 \times 4}{11.5}$	— 0.3200	~~03.2~~

Total Sugar.

1 — 32.5 — 3.64

2 — 42.6 — 4.771

3 — 33.9 — 3.796

4 — 22.6 — 2.531

5 — 29.9 — 3.349

6 — 42.8 — 4.793

7 — 42.6 — 4.771 5.07

Butane diol

1 — 0.0562 — .2271

2 — 0.0214 — 0.0865

3 — 0.0250 — 0.101

4 — 0.0540 — 0.2182

5 — 0.0690 — 0.2787

6 — 0.0150 — 0.0606

7 — 0.0054 — 0.0218

Seed Culture:

Acid.	R. Sugar	Total	Butanediol
0.4	6.1	10.5	0.1140
$\frac{0.0008}{4}$	$\frac{0.6831}{4}$	$\frac{1.176}{4}$	$\frac{0.4606}{4}$
0.0002	0.171	.299	0.115

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Acid

I 0.2 – 0.0006

II 0.2 – 0.0004

Rest alkaline.

Red. Sugar.

I $\frac{200 \times 0.0046}{19.2}$ – 0.048 1.48

II $\frac{200 \times 0.0046 \times 4}{22.2}$ – 0.166 1.66

III $\frac{\quad}{9.0}$ – 0.409 4.09

IV $\frac{\quad}{4.6}$ – 0.8 8.0

V $\frac{\quad}{10.}$ – 0.3681

Total Sugar.

1) 6.0 – 0.6720 0.59

2) 17.0 – 1.904 1.~~9~~5 475

3) 37.4 – 4.189 2.95

4) 53.3 – 5.969 5.9

5) 26.3 – 2.945 2.95

Butane diol:

1) 0.0620 – 0.2505

2) 0.0700 – 0.2828

3) 0.0174 – 0.0702

4) 0.0584 – 0.2359

5) 0.0054 – 0.0218

Same seed as previous one.

0.299
1.475
1.774
2.95
~~+ 425~~
~~.425~~
3.249

5.9
.299
6.199

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Acid

I 0.9 — 0.0018

II 0.25 — 0.0005

III 0.8 — 0.0016

IV 0.3 — 0.0006

V 0.5 — 0.001

SC — 0.4 in 50 — 0.0008

Red Sugar

I 10.3 — $\frac{200 \times 0.0046 \times 4}{10.3}$ = 0.353 — 3.53

II 10.5 — 0.351 — 3.51

III 10.5 — 0.351 — 3.51

IV 11.0 — 0.335 — 3.35

V 10.1 — 0.364 — 3.64

VI S.C. — nil

5.28

Total Sugar

I 50 — 5.601 — 5.13

II 23.6 — 2.643

III 41.2 — 4.614

IV 40.6 — 4.546

V 15.6 — 1.747 — 4.77 .512

5.282

SC/4 — 18.3 = 2.049

4

0.512

Butane diol

I 0.008 — 0.032

II 0.0136 — 0.055

III 0.0558 — 0.225

IV 0.0380 — 0.154

V 0.0020 — 0.081

VI 0.0090 — $\frac{0.036}{4}$

= 0.009

200 x .046 x 4

18.4

.046 x 4

.184 x 200 — 36.8

10.3

3680 — 3.

Molasses Butanol Cultures. Control – 10.2.

	Red Sugar.		Total Sugar
9	– 1·983	9 –	5·124
15	85·2 ×0·0028×20	15	114·4
17	79·7	17	150
18	116·8	18	172
21	85·5	20	79·9
21	79·1	21	116.

Total Acid. All calcld for half ~~it~~ litre results

1	15·2 / 4·16 / 20·8 — 7.6) 3.04 —	200 c.c ~~0.0015~~	200 c.c 0.006	100 c.c ~~for 0.001 × 8~~ = 0.0104 0.003×8 0.0042
2	– 10.4) —	~~0.002~~	0.0083	
3	9.44 / 4 7 2 / ~~23,6~~) —	~~0.005~~	0.019	0.0095
4	5·0	– ~~0·01~~	0·01	0·005
5	5·7	~~0·0114~~	0·0114	0·0057
6	6·0	~~0·012~~	0·012	0·006

Volatile acid. .52

1.	~~2.8~~ 2.6 1.3		~~0.0002~~	0.001	0.0005
2.	~~2.3~~ 2.52 6.3		0.0013	0.005	0.0025
3.	~~27.0~~ 5.32 13.3		0.0027	0.011	0.0055
4	5.3	0.0116		0.0116	0.0058
5	3.0	0.006		0.006	0.003
6	2.8	0.0056		0.0056	0.0028

After oxidn. with Barry

5.72 ~~28.6~~	1. 14.3	(.46)	0.0114 − 0.001 =	.0104	0.0052
12.44 ~~62.2~~	2. 31.1	(.88)	0.0249 − 0.005 =	.0199	0.00995
12.04 ~~60.2~~	3) 30.2	0.55	0.0241 − 0.011		0.0131
	4) 12.6	0.588	0.0252 − 0.0116		0.0066
	5) 12.8	0.86	0.0256 − 0.006		0.0136
	6) 6.7	0.34	0.0134 − 0.0056		0.0068
			0056 / 078		0.0196
					0.0098
					0.0078
					0.0039

Sugar Cultures

Red Sugar

17 — 23.4 × 20 × 0.0028

19 — 32.4

22 — 8.9

Volatile Acid in c.c

1) —

2) 0.6 0.0006

3) 0.9 0.0009.

Total Acid

1) 0.6 0.0006

2) 0.6 0.0006

3) 1.5 0.0015

M_{20} N_{2} C_{3}.

Total Sugar

1) 40.1

2) 42.6

3) 39.7

After oxidn

Calculate & tabulate

1) 6.0 0.006 − 0.006 = 0.53g

2) 9.0 0.009 0.79

3) 9.6 0.0096. 0.85

x Sec.c $\equiv 0.6 \times N/20$

$x = \frac{0.6}{20 \times 5} = 0.006.N.$

$\frac{0.009 \times 88}{48}$ $\frac{72}{72}$ $\frac{792}{}$

$\frac{48}{0.528}$ 4

$\frac{.0096 \times 88}{768}$

$x \times Sec.c = \frac{4.5 \times N}{20}$ $\frac{768}{844}$ $\frac{32.5 \times N}{5 \times 20} = 0.325$

$= 0.0325$

0.065

$x = \frac{4.5 \times N}{5 \times 20} = .045$

0.0045 in 100 c.c

.009 200.

Massen. Total Acid

= first now

· (gerade, jetzt)

normal S. V. O. Er lernt Deutsch

(interrogative sentence) V. S. O Lernt er Deutsch?

Int. Pronoun, Verb. Subj. Obj.

— · —

lang	länger	der die das	längste
kurz	kürzer		kürzeste
groß	größer		größte

a u o — broad vowel e i — narrow

a u o if broad vowels are followed by narrow ones, they are unlauted generally.

kalt	kälter	kälteste
klein		kleinste
dick		dickeste, dickste
		leichste
gut	besser	beste
		weißeste
		schwarzeste

Glycerine cultures. Alkali $\frac{N}{20}$.

Red sugar present.

1) 3.95 × 0.0028 × 20 × 2 = 0.442
2) 2.45 0.27
3) 2.45 0.27
4) 2.55 0.29
5) 13.8. 1.55

Total.

1) 1.1 — 0.12
2) 2.45 — 0.27
3) 0.9 — 0.10
4) 0.8 — 0.09
5) 9.9 — 1.11

Acid in c.c.

1) 4.5 — 0.009
2) 3.5 — 0.007
3) 2.5 — 0.005
4) 0.45 0.0009.

Butane dil.

5) 0.006 × 4.04 — 0.024

V. 0.0034. 0.014

V H − 0.009 — 0.036

— X —

Concentration Cultures. no/: 2

Acid

3) 0.7 — 0.0014

4) 32.5 0.065

5) 117.0 0.234

$\frac{234 \times}{5 \times 20}$

14.3 = 0.049

100 = $\frac{100 \times 0.049}{14.3}$ ×

Red sugar.

250.c → 100.c

5c.cA + 5c.cB = ① 14.3 $\frac{100 \times 0.049 \times 8}{14.3}$ — 2.74

{0.049g. sucrose} ② 20.6 $\frac{39.2}{20.6}$ — 1.42

③ 10.5 $\frac{39.2}{10.5}$ → 3.73

④ 6.7 $\frac{4.9 \times 8}{6.7}$ → 5.52

⑤ 5.1 $\frac{4.9 \times 8}{5.1}$ → 7.69

⑥ 10.4 $\frac{100 \times 0.049 \times 8}{10.4}$ — 3.77

Total Sugar

① 21 c.c × 20 × 0.0028 × 2 — 2.35

② 33.1 c.c " — 3.71

③ 52.6 — 5.89

④ 73.8 — 8.27

⑤ 80.4 — 9.00

⑥ 43.5 — 4.87

X ⟶ negative result.

X_H. ① —

② 0.008 — 0.032

③ 0.0336 — 0.136

④ 0.0262 — 0.106

⑤ 0.0528 — 0.213

⑥ 0.0170 — 0.069

X_B ① 0.0380 × 4.04 = — 0.154

② 0.0060 × 4.04 — 0.024

③ 0.0190 — 0.077

④ 0.0384 — 0.155

⑤ 0.0290 — 0.117

⑥ 0.0070 — 0.028

*D. ① - 0.0276 — 0.112

② - 0.0006

③ - 0.0396 0.149

④ - 0.0174 0.070

⑤ - 0.0104 0.042

⑥ - 0.0040 0.016

Effect of $ZnSO_4$ on no: 2.

Acid @ Alkaline.

Red sugar. ① 6.0 39.2/6 = 6.53

② 6.6 5.94

③ 7.1 5.52

④ 6.4 6.13

⑤ 6.6 5.94

⑥ 10.1 3.88

⑦ 6.4 6.13

Total Sugar ① 57.1 × 20 × 0.0028 × 2 — 6.4

② 52.0 — 5.82

③ 51.2 5.73

④ 40.8 4.57

⑤ 52.9 5.93

⑥ 40.3 4.51

① 43.0 4.82

Concn Cultures no/14

Acidi sc. ① 0.3 — 0.0006
② 0.6 0.0012
③ 4.6 0.0092
④ 86.5 0.1730
⑤ 150 0.30
⑥ alk. — 14.9 cc

1 cc = 0.0028 × 2.
14.9 × 0.0028 × 2 × 40

Red Sugar
✓ ① 21.7 39.2/21.7 — 1.81
② 7.9 39.2/7.9 — 4.96
③ 5.3 39.2/5.3 7.4
④ 4.2 39.2/4.2 — 9.33
⑤ 3.2 39.2/3.2 12.25
⑥ 10.6 39.2/10.6 3.7

·112 × 14·9
·298
149
149
1·6688

Total Sugar ① 14.9 × 2 × 0·0028 × 2. — 1.67 = 3.34
② 34.3 — 3.84
③ 44.6 — 4·995
④ 59.4 — 6.65
⑤ 71.2 — 7.97
⑥ 35.8 — 4.01

X. ① 0.006 — .024

② 0.002 — 0.008

③ 0.0160 — 0.065

④ 0.0240 — 0.097

⑤ 0.0344 — 0.137

⑥ 0.008 — 0.032

X_H ① − 0.0020 — 0.008

② − 0.0060 — 0.024

③ − 0.0200 — 0.081

④ − 0.0230 — 0.093 − 0.093

⑤ − 0.0350 — 0.141

⑥ − 0.0130 — 0.053

X_D ⑤ — 0.001 — 0.004

X_B. ② − 0.003 × 4.04 — 0.012

③ − 0.01 × 4.04 — 0.040

5 c.c. + 5 c.c = .049.

21.7 = .049 100 = .049 × 100 × 8 / 39.2

25 = .049 × 4 × 8 / 176 × 8 4

200 = 1408

Red Sugar

SMART

28 → 100 ① — 30.6 — 39.2/30.6 ~~7.28~~ 1.28

② — 6.7 39.2/6.7 — 5.85 5.85

③ — 6.3 39.2/6.3 — 6.22

④ — 3.4 39.2/3.4 — 11.53

nodule ⑤ — 26.8 39.2/26.8 — .365 26.8 − 0.049 ~~9.800~~ 26.80 .2

⑥ — 45 c.c — 39.2/45 — 0.87

Total Sugar

① — 52.7 × 20 × 0.0028 × 2 — 5.90

② — 44.2 — 4.95

③ — 45.2 — 5.06 5.06

④ — 61.8 — 6.92

⑤ — 39.6 — 4.44

⑥ — 47.2. — 5.29

X ① — 0.0021 — 0.508

② — 0.0036 — 0.015

③ — 0.0090 — 0.036

④ — 0.0050 — 0.020

⑤ — 0.0058 — 0.023

⑥ — 0.0154 — 0.062

(XH) ① — —

② — 0.0180 — 0.040

③ — 0.0110 — 0.044

④ — 0.0054 — 0.022

⑤ — 0.0130 — 0.053

⑥ — 0.0170 — 0.069

(XD) ① —

② — 0.0090 — 0.036

③ — 0.0030 — 0.012

④ — 0.0100 — 0.04

⑤ — 0.0040 — 0.016

⑥ — 0.0254 — 0.10

Pentamedin

① — 0.0006 × 4.04. — 0.002

② — 0.0248 — 0.10

③ — 0.0340 — 0.137

④ — 0.0316 — 0.128

⑤ — 0.0294 — 0.119

⑥ — 0.0300. — 0.121

National Research Fellowship.

200/-

$HgCl_2$

$C_4H_{10}O \rightarrow C_3H_7COOH$

$CH_3 \cdot CH_2 \cdot CH_2 \cdot COOH$

$C_4H_{10}O \rightarrow C_4H_8O_2$

48
10
16
---
74 → 88

48 + 10 + 16 = 74 → 48 + 8 + 32 = 88

1E — 88 → 74

0.77gm

$\frac{74 \times 0.0104}{296}$

Ramgam 740

Kamakshi 7696

74 × 0.199 = 666, 666, 74 → 1.4726

0.00995 × 88 → 7960, 7960 → 0.87560

416, 416 → 4576

0.0052 × 88 =	0.46
0.00995 × 88 =	0.88
0.0066 × 88 =	0.55
0.0068	0.598
0.0098	0.86
0.0039	0.34.

498, 498 → 5478

68 × 88 → 544, 544 → 5984

98 × 88 → 784, 784 → .8624

39 × 88 → 312, 312 → 3432

28.2 / 0 — Thinking life a bore
Took some dil H_2SO_4 KCNS
But his father, an M.D → $HgCNS$ + KCl
Gave him $CaCO_3$
He neutralised it's true
But he is full of CO_2

$\cdot 6$ c.c $N/50 \equiv 5$ c.c $\times x$ — lower layer.

0) 3.95 — 3.95, 4.0 — 21.1, 25.1) 4.0

$x = \frac{7.6 \times \overset{5}{N}}{5 \quad 50} = \frac{7.6}{250} N$

4) 8.5, 12.5 — 12.5, 21) 8.5

Stan

1N — 1L — E

$\frac{7.6 \times N}{250}$ — 1L — $\frac{7.6E}{250}$

100 — $\frac{7.6}{250 \times 10}$

200

·4.0 / ·470

8.5) 400, 25, 340, 600, 595, 50 — ·47

$\frac{7.6 \times 2}{10 \times 50 \times 2} = \frac{7.6 \times 2}{1000}$

$7.6 \times 2 \times \frac{N}{50} = 10 \times C$

x. $\frac{7.6 \times 2}{50 \times 10 \times 5}$

$5.0 \frac{N}{20} = 5.$

1L — 1N — 1E

1L — $\frac{N}{20}$ — $\frac{E}{20}$

·1L — $\frac{N}{20} = \frac{E}{20} \times \frac{10}{1000}$

200

1c.c — $\frac{E}{20 \times 1000}$

1L — 1N — 1E

1L — $\frac{N}{50}$ — $\frac{E}{50}$

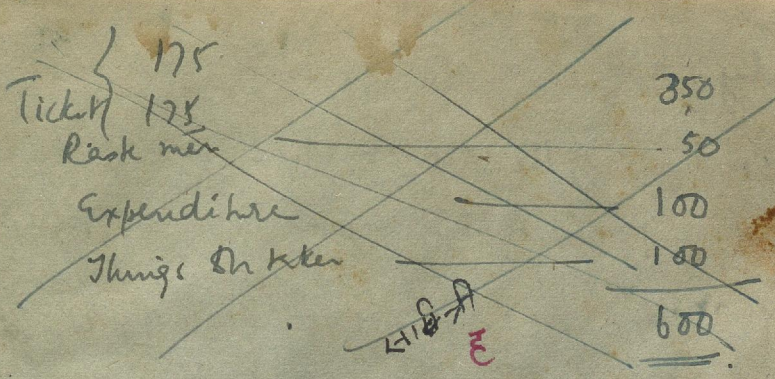
1c.c $\frac{E}{50 \times 1000}$

$\frac{x \times E \times 10}{50 \times 1000} = \frac{x}{1250}$ (500, 250)

$x = \frac{x \times E}{20 \times 1000} \times \overset{2}{10} = \frac{x2}{1000} =$

$\frac{\cdot 015 \times 2}{5}$ (·003) $= 0.006$

31.3160
9.808
41.1260



Strains being studied

2] A. Aerogenes

4] B. ~~Subtilis~~ Polymyxa

8] S. Marcescens

10] B. Subtilis

24] Aeromonas hydrophila.

14] Dhai yeast.

To be Studied

15] Cl. Butyricum.

17] Cl. Acetobutylicum

21] Cl. pasteurianum

22] Cl. Butylicus.

July

Study of the formation of butanediol & butanol from molasses using different organism

August.

100 c.c — 1g.

4.9 c.c = $\frac{4.9 \times .01}{100}$ = .0049 g.

Same.

September $\frac{1}{100} = .01$

Study of the formn of butanediol from molasses using different strains.

October. opt. conditions

Study of butanediol formation from molasses using A. aerogenes.

November. Study of the optimum conditions for the formn of B. butanediol from molasses by B. Polymyxa

160 - 90

$x \times 90 = 10 \times 70$

$90 + 10 = 70 + 30$

$x = \frac{100 \times 70}{90}$

10 - 90

70 m. -70

78 c.c → 100

0.53 — 1000 — N

5.3 — 1000 — N/10

0.53 — — N/100

2.65 — — N/20

5.3/2 — 500 — N/10

5.3/2×5 — 500 — N/50

0.53 — 500 — 0.53×2/53 = N/50

150 50

10	65	10
20	55	20
25	50	30
35	40	40
50	25	50
75	—	60
		75

0.17×20 / 10 2 / 34 / 5.4 2

25	125
50	100
75	75
100	50
125	25
0.08×150	—

10 → 75 65/5 Control 25 → 100

13 → 10

Apply - albumen on the slid. keep the material on it. Put 10 %. KON. solution & fix.

Upgrade. 50 %. Al
70 %. Al
~~90 %~~.

stain in Borax carmine
keep for 15 minutes. (Do not stain)
~~Keep~~ Differenciate in 70%.

Then dip in ~~Di~~ Picro - indigo carmine. Dip in 90 %. Al then 100 %, Zylol. Put canada Balsm & mount.

Prof. Shiban Lal Saxena M.P.
Parliament House.
New Delhi

315.5

$Ba(OH)_2 + 2HCl \rightarrow BaCl_2 + 2H_2O$

$\frac{315.5}{2}$

157.75

157.75 - 1 l - N

15.775 g. - 1 l. $\frac{N}{10}$

N/20 Baryta.

Dissolve about 35 gm. $Ba(OH)_2$ in 350 ml water (boiling) contained in a flask, then fit the flask with a cork carrying a soda lime tube. and set aside until cold. The excess of baryta crystallises and a clear Sat. soln which about 0.35N is obtained

$K_2Cr_2O_7 + 6FeCl_2 + 14HCl = 6FeCl_3 + 2KCl + 2CrCl_3 + 7H_2O.$

34.648 in 500.

5 c.c ≡ 0.05 g sugar

Bramto Perko

500 ≡ $\frac{0.05 \times 100}{0500}$

311 [redacted]

$K_2Cr_2O_7 + 6FeSO_4 + 7H_2SO_4 = 3Fe_2(SO_4)_3 + K_2SO_4 + Cr_2(SO_4)_3 + 7H_2O$

$K_2Cr_2O_7$ x

$Cu_2O + Fe_2(SO_4)_3 + H_2SO_4 = 2FeSO_4 + 2CuSO_4 + H_2O$

$\rightarrow 2CuSO_4 + 2FeSO_4 + H_2O.$

$Cu_2O \equiv 2FeSO_4 \equiv \frac{K_2Cr_2O_7}{3} \equiv 2CuSO_4$

≡ 〃 ≡ $\frac{294.22}{3}$

71.56 ≡ x/2 ≡ 4.904 ≡ $CuSO_4$

4.904 ≡ 249.56

69.28

63.56
8
71.56

63.56 $5H_2O$
32
64
159.56
90
249.56
4
61.

$Cu_2O \equiv 2CuSO_4$

34.64 g $CuSO_4$ ≡ 5g of sugar

249.66 = $\frac{249.66 \times 5}{34.64}$ = 36.28 g

$\frac{249.6 - 159.6}{34.64 - 4.33} = \frac{4.33 \times 159.6}{249.6}$

$= \frac{4.33 \times 18.3}{5.2}$

= 36.28 g

4.904 = 36.28 g of Sugar

in 1000 c.c = 36.28 g

1 c.c = $\frac{0.03628 \times 1000}{36.28008}$

$\frac{36.28}{1000}$

1 c.c of the dichromate prepared is equal to 0.0363 g of Sugar.

12 (581), ATCC (10132) Clostridium acetobutylicum
12m16

12 (583) ATCC (6013) Clostridium pasteurianum
12m16

19) 12 (380) IAL (15-152) Clostridium Butylicum
12m16

NRRL-
20) B-527 C. acetobutylicum
21) B-598 C. pasteurianum
22) B-592 C. butylicus.
23) B-510 RHG Bacillus polymyxa
24) B-909 Aeromonas hydrophile
25) B-543 Bacillus Subtilis
26) NRRL. B-284 S. marcescens

NRRL. Illinois

27) B. Subtilis

NCL. Poona

NO: 2010.

Instituto Zimotecnico

Microbiologia

28 Bakers yeast Fleischmann Co Bologna

29) S. Cerevisiae

NCL Roma

exact N/10 dichromate soln

4.904 g in 1 l.

31.3176
4.904
36.2216

Titrated with 10 c.c of 1% glucose AR soln.

10 c.c requires 35.2 c.c dichromate ∴ 1 c.c dichromate = 0.0028 g of glucose

200%

गोटि – embroidery.

धोती – Dhoti.

necklace –

31.3180
4.904
36.2220

In the bill of Feb. '57. (1.3.57).

comparative
Study of the formation of 2.3. butane diol in culture of different butanediol fermenting bacteria ~~utilising~~ ~~[illegible]~~ using different carbohydrates and the source of C food.

2.7. dimethyl 1,4 $O_2C_{10}H_4$.

CH_3 1,4 naphtha quinone

2 hydroxy 1,4 "

2,7. dimethyl 1,4 n. q.

Juglone

Phthiocol

β naphthaquinone

2,3. dimethyl 1.4 n.q.

α naphthaquinone.

0·008 × 0·202

16

160

·00161 6 × 20

·03232

·02 × ·202

·0404 × 20

·0808

·055 × ·202

110

1100

·0111 10 × 20

·2222

5gm in 100 c.c

i.e. $\frac{5}{100}$

= ·05

12·7 — 0·05

50 — 50 × 0·05 — 5

12·7

100 × 50 × 0·05 — 100

12·9 × 8

50/12·7

3·7

127) 500 (2

388

1120

177) 500 (2·88

354

1460

1316 5

1440

6

·048 × 1·648

3

13184

6592

0·079104

Na_2CO_3 — 53.

N/20. soln.

53.

53 / 2×2

5.3 / 2×2×10

4) 5.3 (1.325
4
1.3
12
10
8
20

3 - 12 → 9
12 - 12 — 24
12 - 10 — 10
43

11

~~10 - 10 - 24.~~
~~10 - 3 - 2~~

500

1000.

N. / N/20

E / 20

E × 1000 / 20 × 1000

N / 2

N / 20

1c.c

.806 × 40 × 2 / 26 × 1000 (500) 0.0012

.049 × 100 / 04900.

Prepared exact N/20 soln of Na_2CO_3 on 28.1.57. (500c.c) I

Previous soln. II.

10c.c of II × HCl.

4.3 / 6.8) 2.5

10c.c of I × HCl

6.8 / 18.6) 11.8 c.c.

$11.8 \times \frac{N}{20} = 2.5 \times x$

$x = \frac{11.8 \times N}{20 \times 2.5} = \frac{11.8}{50} = \frac{1.18}{5}$ N

= .236 N strength of Na_2CO_3 beginner.

46.1 × 0.028 × 2
46.1 0.056
2766
2305
9.5816

446) 990 (.20
892
800
4

1N — 1 equt — 1 l

0.236 N — 0.236 equt — 1 l.

$\frac{0.236}{1000}$ 1 c.c

= .000236 × 5 5 c.c.

0.001180 — 5 c.c.

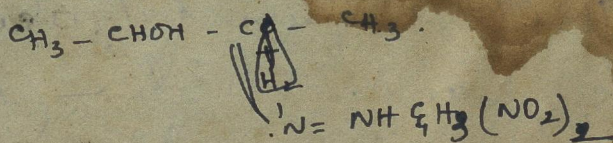
0.00118 is in ~~x~~ c.c.

~~.00~~0 11800 × x — 100

5 c.c ≡ x.

5 × 0.00118 — x.

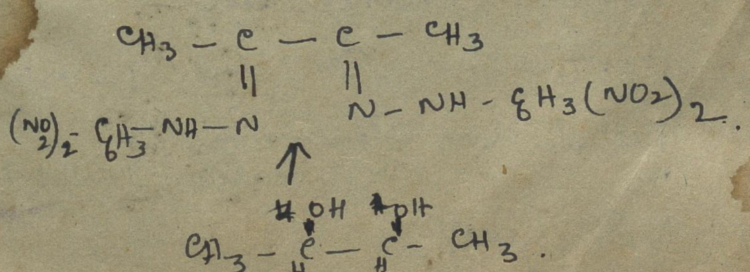
$\frac{.118}{x.}$ = 100



16×12 =	192
8×14 =	112
1×14 =	14
8×16 =	128
	446

48
32
10
90

446 – 90
1 – 0.202



21.11.

(1) # 492 Pseudomonas hydrophila Ottawa

(2) M-148 Aerobacter aerogenes "

(3) # 474 Aerobacter aerogenes "

(4) C-3(2) Bacillus Polymyxa "

(5) B-2 Bacillus Subtilis "

(6) S-29 Serratia marcescens "

(7) B. Subtilis. } Poona

(8) S. marcescens } "

2nd Ins received on 8th Jan 1957.

(9) Clostridium butyricum (Prazmowski) From National Collection of Dairy Bacteria

(10) Bacillus [illegible] Ottawa (Ehrenberg) Jorgensen

(11) Serratia marcescens — "

(12) Aerobacter aerogenes (Kruse) "

(13) Myrothecium ~~Cellulosolvens~~ verrucaria WSDA 1334 Ottawa

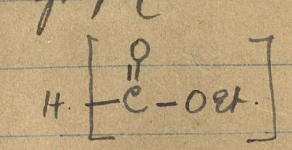
(14) Dhar Yeast

(15) Clostridium Butyricum 6084 England

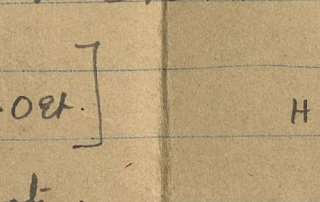
(16) Bacillus Subtilis

Increase of reducing property:

Other than formyl group formate, lactate, chloroform, iodoform, uric acid etc. other compounds also have properties of reducing Fehling solution. That is the following arrangement of hydrogen makes the hydrogen atom so labile as it acts as reducing grp. (as it cateches one Oxygen and leaves OH grp)



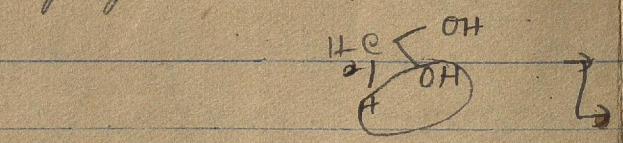
Formate.



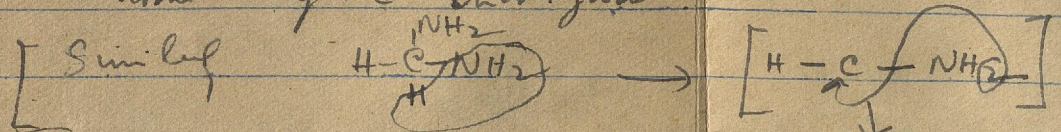
Lactate.

i.e. if a carbonyl oxygen or ester grp is attached to the C carrying hydrogen atom then hydrogen is reducing. Or. But as Ketene $CH_2=CO$ does not have such reducing property. Carbonyl grp oxygen and alkyl attached to the carbon carrying hydrogen makes an ideal aldehyde. Halogen atoms, weak ortho + para directing grps, also make the hydrogen attached to the α carbon atoms reducing. -OH, $-NH_2$ are strong o & p directing grp yet only one of such grp does not makes the hydrogen atom sufficiently active as to act as reducing H of course the compds containing two or more OH NH_2 grp attached to one carbon atom are so unstable that

influence on the hydrogen attached to this carbon atom is not known. Perhaps it is due to the activation of hydrogen that H_2O & NH_3 are illi-mitably such couples.



Just by the way this mechanism of dehydration of dihydroxy compound having the OH groups on the same carbon atom may be envisaged.



$H_2C\{_2 + NH_2 \rightarrow CH_2(NH_2)_2$

$CH_2 = NH$.

reaction may be investigated. Methylenimine has not been known so far. But hexamethylene tetramine is known by the following reaction.

$$CH_2 = O + H_2NH \rightarrow CH_2 = NH.$$

Compounds having one OH & NH_2 attached to the same carbon atom are rare (unknown aminophenol).

$OH - CH_2 - NH_2$ though if it contains with H_2O

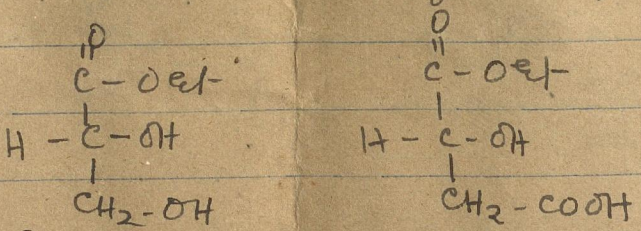
$$OH - CH_2 - NH_2 + H.OH \rightarrow [OH\,CH_2 \cdot NH_3]\,OH$$

and compounds of this skeleton are of very great biological importance as Colamine and Choline.

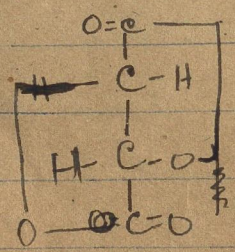
$CH_2 NH_2 \cdot CH_2 OH$ $\qquad$ $OH(CH_3)_3 N \cdot CH_2 \cdot CH_2 OH$

So to activate the hydrogen to act as reducing hydrogen with Fehling solution the carbon atom should have a carbonyl oxygen & a ~~alkoxyl~~ alkoxy group as in formation a strong a electron repelling group, a weak electron repelling gr and a weak electron attracting attracting group as in lactate as OH, $CH_3-\overset{O}{\overset{\|}{C}}-OCH_3$, ($-CH_3$ can be replaced with $-CH_2X$ group which is a little ~~more~~ weaker electron repelling group)

Thus a compound with the following str. will have ready hydrogen.

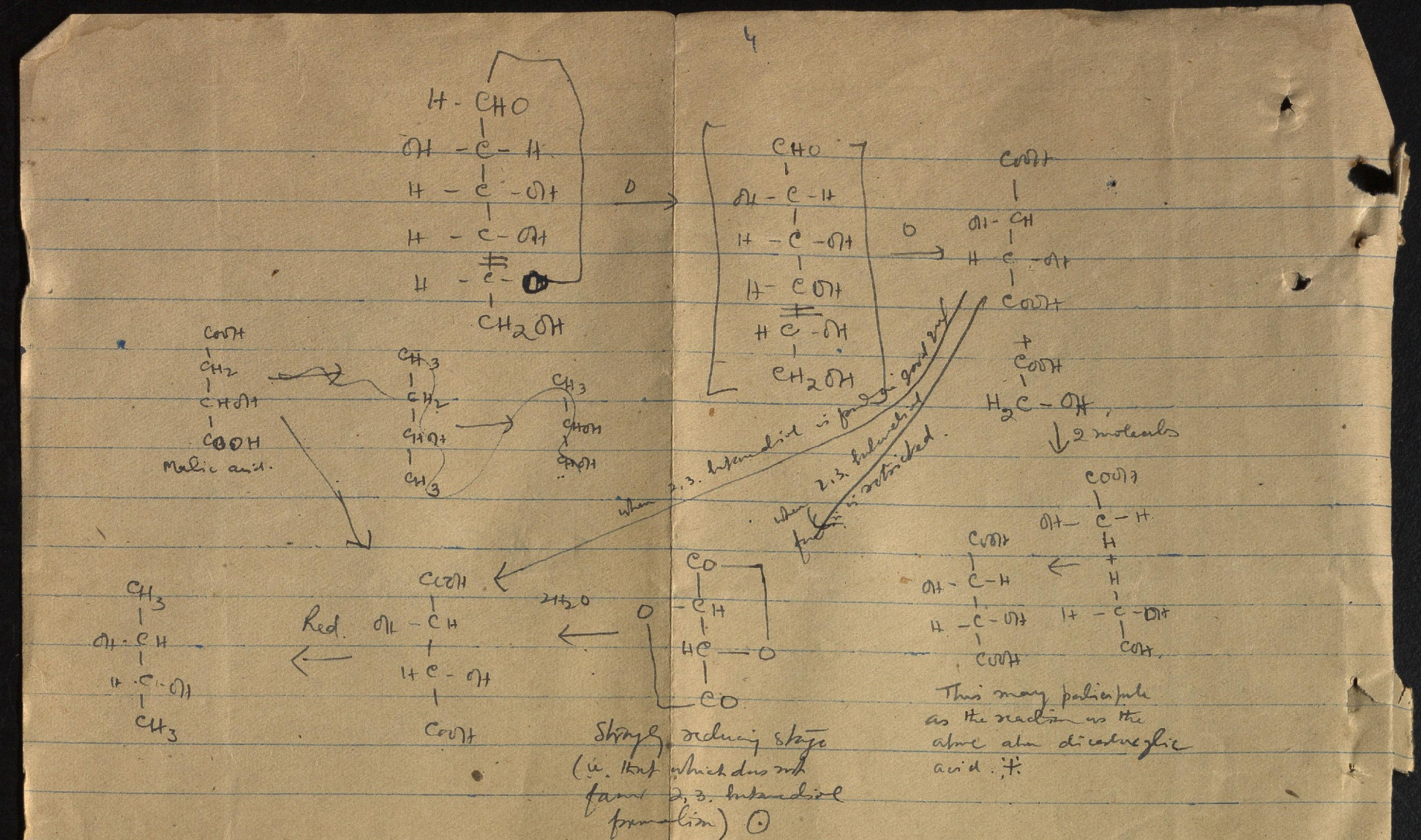


But $-\overset{O}{\overset{\|}{C}}-OCH_3$ group may be replaced by $-\overset{O}{\overset{\|}{C}}-O-$ lactone group. Thus to explain ~~the~~ group reducing power we can possibly think on the following formulae.

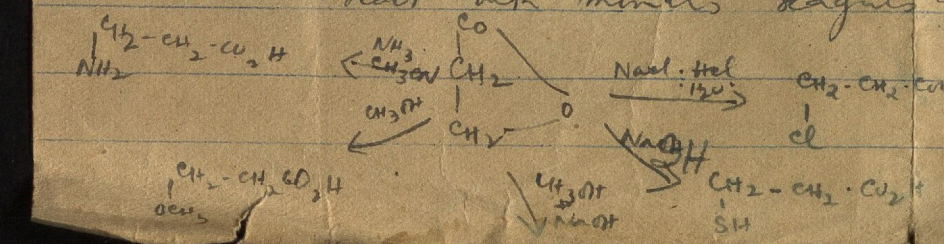


This double propyl lactones and single propyl lactones have been synthesized in ~~the~~ my method and are ~~effective~~ active compounds.

This double propyl lactone can formed by the following reactions:



⊙ The reducing property is destroyed on boiling with HCl. Butyrolactone or β-lactones as they are known can react with mineral reagents as follows:



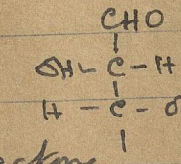
Thus it will be interesting to investigate whether the ~~dilactone~~ dipropriolactone as hypothesised is actually formed. If so [illegible]

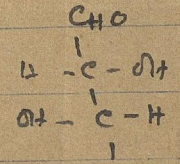
can be formed by

1. Loss of reducing property on
   (a) boiling with HCl –
   (b) Boiling with CH_3OH.
   (c) Boily with $CH_3OH + NaOH$.
   (d) NaSH.

   } see the corresponding compd. formed a part of the reagent is used up & it will decrease in strength.

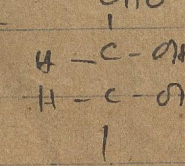
2) Formation of the reducing compd. of altrose, glucose, idose, and galactose as used as substrate (provided they are utilised by the organism in consideration) because they contain

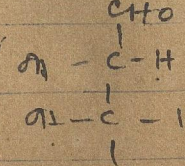




group which can arise from dipropiolactone.

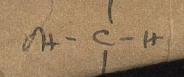
allose, mannose, gulose, and talose will produce only half as much reducing property if they are utilised by the organism under consideration) because that have





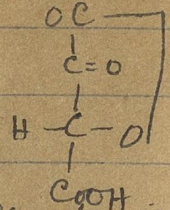
group and in these cases only of single β-propiolactone can be formed and not the double lactone due to steric hinderance

3. If the fructose is used which has



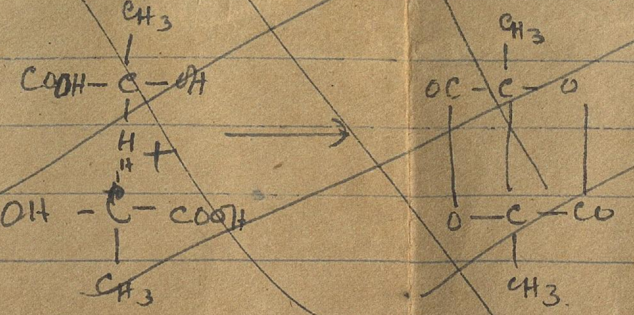
group at

one terminal the above reacts will form ring



Compound will form which will have lesser reducing property.

~~.43 The β propiolactone may be formed from lactic acid also as follows~~



~~Quantitative the~~

Investigate the ~~...~~ formation 2.3, butane diol ~~and~~ employing lactic acid and glycolic acid as substrate.

Intp.

Instructions

1) Most of the experiments should contain sucrose as substrate.
2) ~~Use Gluc~~ Observe the formation of 2,3 butanediol in presence of tartaric acid and Glycolic a.
3) See the point are on page 5 and perform experiments.
4) See and verify the points 2 and 3 on the page 5.

1. Study the fermentation of 2-3 butanediol fermentation [illegible] yeast without $ZnSO_4$, 0.05 per 400 c.c 0.1/400 c.c 0.15 and 0.2 with mineral nutrients as usual

2. Investigate the formation of reducing material in Serratia marcescens & Aerobacter aerogenes, containing sucrose as source of carbon and 1% milk.

Prepare [illegible] culture. pH – 7.
Sucrose 5% (pure). ($MgSO_4$ 0.08, $(NH_4)_2SO_4$ ·17 gm, Phosphate 0.3 g per litre)
milk. 1%; (control without milk) and 2%;
A. Aerogenes :– culture as above, without milk, (1) with 0.3 gr. of phosphate [illegible], (2) with 1% $CaCO_3$, (3) with 0.01 gm $CuSO_4$.

In the case of S. marcescens large quantity of total sugar is [illegible] when as the reducing sugar remains the little. In case of A. aerogenes in their culture large quantity of reducing sugar are produced whereas the total sugar remains same.

To estimate the sugar and all reducing compounds by –CHO group.

To estimate the volatile reducing materials, as acetone, acetaldehyde & [illegible] alcohols etc.

To study why the reducing property is destroyed on boiling with HCl in certain cases.

Following estimations should be done in each case:

1, Reducing sugar. 2 Total sugar
3, Acid formed.
4, Estimate the =CO group present in the fermented solution.

(A) Take 5 c.c. of the solution and to it add 2-4 dinitrophenyl hydrazone in HCl separate the ppt. dry weigh.

(B) Take 5 c.c. of fermented sol. in a distilling flask add 15 c.c. of water & distill till only 5 c.c. are left. Collect the filtrate in hydrazine sol. filter, the precipitate, dry & weigh.

(C) Take 5 c.c. of the fermented liquid hydrolyse it with HCl and add phenyl hydrazine – separate the ppt. & [illegible]