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APPENDIX-I

Distillery yeast biomass waste sample analysis

Estimation of Calcium

1. Sodium hydroxide 1N: 40g of sodium hydroxide was dissolved in one liter of distilled water.

2. Murexide indicator: 200mg of the dye was ground with 100mg of sodium chloride.

3. Standard EDTA titrant, 0.02N.

Estimation of Chloride

Chloride free double distilled water was used for all the reagents

1. Standard silver nitrate titrant, 0.0282N: 4.791g of silver nitrate was dissolved in one litre of distilled water. Standardized it against 0.02N sodium chloride solution. 1.0ml of exactly 0.02N AgNO₃. AgNO₃ = 1.0mg of chloride.

2. Standard sodium chloride titrant, 0.0282N: 1.648g of sodium chloride was dissolved in one litre of distilled water 1.0ml=1.0mg of chloride.

3. Potassium chromate indicator solution: Dissolved 25g in 100ml of distilled water. Added silver nitrate solution drop wise until a slight red precipitate was formed. Allowed to stand for twelve hours. Filtered and made upto 500ml with distilled water.
4. Aluminium hydroxide suspension: Dissolved 100g of Aluminium ammonium sulphate in 1000ml distilled water. Warmed to 60°C and added 55ml concentrated ammonia solution. Allowed the precipitate to settle for about an hour. Washed by decantation with distilled water to make the precipitate from chloride. Checked it by treating portion of the decanting every time with silver nitrate solution. After the precipitate was free from chloride, diluted it to 1000ml with distilled water.

**Estimation of Sodium and Potassium**

1. Sodium stock solution: 2.524g of sodium chloride was dissolved in deionised water and made upto one liter. 1.0ml = 1.0mg of sodium.

2. Potassium stock solution: 1.907g of potassium chloride was dissolved in deionised water and made upto one liter. 1.0ml = 1.0mg of Potassium.
MEDIA COMPOSITION

1) Nutrient Agar (pH 6.8)

Peptone : 50g
Beef extract : 30g
Sodium chloride : 20g
Agar : 15g
Distilled water : 1000ml

2) Rose Bengal Agar (pH 7.2)

Mycological peptone : 5.0g
Glucose : 10.0g
Potassium dihydrogen phosphate : 1.0g
Magnesium sulfate : 0.5g
Rose Bengal : 0.05g
Chloramphenicol : 0.1g
Agar-agar : 15.5g
Distilled water : 1000ml

3) Kenknight’s Agar (pH 7.2)

Glucose : 1.0g
KH₂PO₄ : 0.1g
NaNO₃ : 0.1g
KC1 : 0.1g
(NH₄)₂SO₄ : 0.1g
MgSO₄.7H₂O : 0.1g
Agar : 15.0g
Distilled water : 1000ml

4) Kings B medium (pH 7.0)
Glycerol : 10ml
Proteose peptone : 20g
Dipotassium hydrogen orthophosphate : 1.5g
Magnesium chloride : 1.5g
Ampicillin : 100ppm
Chloramphenicol : 50ppm
Cycloheximide : 10ppm
Distilled water : 1000ml

5) M9 Minimal Salts Medium
Disodium hydrogen phosphate : 6 g
Potassium dihydrogen phosphate : 3 g
Ammonium chloride : 1 g
<table>
<thead>
<tr>
<th>Ingredient</th>
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<tbody>
<tr>
<td>Sodium chloride</td>
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<tr>
<td>Magnesium sulphate</td>
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<tr>
<td>Distilled water</td>
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<tr>
<td>Calcium chloride</td>
<td>0.01 g</td>
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6) **Acetate Minimal Medium**

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<td>Dipotassium hydrogen phosphate</td>
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<tr>
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<td>pH</td>
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