Annexure 1: Calibration curve for estimation of Acetylene and Ethylene using gas chromatography

![Calibration curve](image)

**Fig. A1**: Calibration curves of gas chromatographic response for (a) acetylene (99.6%) and ethylene (99.96%) expressed in injection volume (µl) and (b) ethylene expressed in nmoles

**Explanation**

All gas calculations are done at normal temperature and pressure (NTP) (pressure = 760 mm Hg or 1 atm; temperature = 273° K). Convert 1 ml of ethylene to ml of ethylene at NTP using the gas law relationship:
Where \( P_1 = 760 \text{ mm Hg}, V_1 = \text{unknown}, T_1 = 273° \text{ K} \)

\[
P_2 = 760 \text{ mm Hg}, V_2 = 1 \text{ ml}, T_2 = (273° + 23°) \text{ K}
\]
and \( P_2 = 760 \text{ mm Hg}, V_2 = 1 \text{ ml}, T_2 = (273° + 30°) \text{ K} \)

- 1 ml of ethylene at RT and 1 atm (760 mm Hg) = 0.922 ml at NTP
- 1 ml of ethylene at RT (30°C) and 1 atm (760 mm Hg) = 0.900 ml at NTP

**Conversion of ethylene (ml to nmoles):**

1 mole of \( \text{C}_2\text{H}_4 \) at NTP occupies 22.4 liters.

0.922 ml of ethylene at NTP = 4.12 x 10^{-5} \text{ mol}

\[
= 0.0000412 \text{ moles} = 41200 \text{ nmol}
\]

0.900 ml of ethylene at NTP = 4.01 x 10^{-5} \text{ mol}

\[
= 0.0000401 \text{ moles} = 41000 \text{ nmol}
\]

1 ml of 1% ethylene injected into the GC = 412 nmol

1 ml of 1% ethylene injected into the GC = 401 nmol

1 ml of 10% ethylene injected into the GC = 4010 nmol

100 µl of 10% ethylene injected into the GC = 401.0 nmol

100 µl = 401.0 nmol = (peak area)

200 µl = 401.0 x 2 nmol = 802 nmol = (peak area)

300 µl = 401.0 x 3 nmol = 1203 nmol = (peak area)

400 µl = 401.0 x 4 nmol = 1604 nmol = (peak area)

500 µl = 401.0 x 5 nmol = 2005 nmol = (peak area)
### Annexure 2: Pattern of fatty acid methyl esters of *Halomonas* spp. and its quantum

<table>
<thead>
<tr>
<th>RT</th>
<th>Response</th>
<th>Ar/H</th>
<th>RFact</th>
<th>ECL</th>
<th>Peak Name</th>
<th>Percent</th>
<th>Comment 1</th>
<th>Comment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.7437</td>
<td>1.65E+6</td>
<td>0.008</td>
<td>6.6657</td>
<td></td>
<td>&lt;min rt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.7522</td>
<td>1.09E+6+9</td>
<td>0.019</td>
<td>6.7292</td>
<td>SOLVENT PEAK</td>
<td></td>
<td>&lt;min rt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.9935</td>
<td>3.60</td>
<td>0.008</td>
<td>8.5248</td>
<td></td>
<td>&lt;min rt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0289</td>
<td>440</td>
<td>0.011</td>
<td>8.9796</td>
<td></td>
<td>&lt;min rt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.1308</td>
<td>891</td>
<td>0.018</td>
<td>1.3723</td>
<td>9.5662</td>
<td>unknown 9.560</td>
<td></td>
<td>ECL deviates 0.006</td>
<td></td>
</tr>
<tr>
<td>1.1888</td>
<td>1783</td>
<td>0.012</td>
<td>1.3171</td>
<td>10.0019</td>
<td>10.0</td>
<td>0.23</td>
<td>ECL deviates 0.002</td>
<td>Reference 0.014</td>
</tr>
<tr>
<td>1.2698</td>
<td>1148</td>
<td>0.015</td>
<td>10.4770</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.3597</td>
<td>1612</td>
<td>0.012</td>
<td>1.2041</td>
<td>11.0024</td>
<td>11:0</td>
<td>0.19</td>
<td>ECL deviates 0.002</td>
<td>Reference 0.007</td>
</tr>
<tr>
<td>1.4550</td>
<td>24489</td>
<td>0.009</td>
<td>1.1601</td>
<td>11.4548</td>
<td>10:30H</td>
<td>2.77</td>
<td>ECL deviates 0.007</td>
<td></td>
</tr>
<tr>
<td>1.4863</td>
<td>533</td>
<td>0.013</td>
<td>1.1431</td>
<td>11.6030</td>
<td>12:0 iso</td>
<td>0.06</td>
<td>ECL deviates -0.018</td>
<td>Reference -0.017</td>
</tr>
<tr>
<td>1.5316</td>
<td>2131</td>
<td>0.009</td>
<td>1.1251</td>
<td>11.8180</td>
<td>unknown 11.825</td>
<td></td>
<td>ECL deviates -0.007</td>
<td></td>
</tr>
<tr>
<td>1.5706</td>
<td>61795</td>
<td>0.008</td>
<td>1.1101</td>
<td>12.0025</td>
<td>12:0</td>
<td>6.69</td>
<td>ECL deviates 0.002</td>
<td>Reference 0.001</td>
</tr>
</tbody>
</table>
**ANNEXURES**

Studies on some aspects of nitrogen fixation by microbes from alkaline and saline soils


<table>
<thead>
<tr>
<th>ECL Deviation</th>
<th>Reference ECL Shift</th>
<th>Number Reference Peaks</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.006</td>
<td>0.010</td>
<td>20</td>
</tr>
</tbody>
</table>

Total Response: 1139129  
Total Named: 1121068  
Percent Named: 98.41%  
Total Amount: 1038055  
Matches:

<table>
<thead>
<tr>
<th>Library</th>
<th>Sim Index</th>
<th>Entry Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>RTSBA6 6.00</td>
<td>0.680</td>
<td><em>Halomonas</em> spp.</td>
</tr>
<tr>
<td></td>
<td>0.462</td>
<td><em>Pseudomonas stutzeri</em> (<em>P. perfectomarina</em>)</td>
</tr>
<tr>
<td></td>
<td>0.220</td>
<td><em>Pantoeaagglomerans</em>-GC subgroup C (<em>Enterobacter</em>)</td>
</tr>
</tbody>
</table>

Annexure 3: Calibration curve for estimation of IAA

Fig. A1: Calibration curve for estimation of IAA (slope = 4.1309) estimated using standard IAA (Loba Chemie, Mumbai) using method described by Brick et al. (1999)

\[ y = 4.1081x \]
\[ R^2 = 0.9819 \]
Annexure 4: FAME analysis report of RAP3 strain.

Royal Life Sciences Pvt.Ltd.
Affiliated to MIDI Sherlock, USA

Plot No. 41, Samrat colony, W. Marredpally, Secunderabad -500 026, (Twin city of Hyderabad), INDIA.
Tele Fax: + 91-40- 65795687 / 27803738 / 27703154
Email: info@royalgroupinfo.com Site rwww.royalgroupinfo.com

ANALYSIS REPORT

ISSUED TO: R. D. Shelar,
Research Scholar, Issue Date: 02/04/2012
Dept. of Microbiology,
Z. B. Patil College, Deopur,
Dhule - 424 002

Report Number: RLS/REP/046

SAMPLE DETAILS

Sample Received Date: 24/03/2012
Sample Ids: 1. RAP 3

Service Requested for: FAME Analysis

No. of Samples: 1

Growth Conditions: Aerobic Culture

RESULT

<table>
<thead>
<tr>
<th>S.No</th>
<th>Sample Id</th>
<th>Analysis</th>
<th>Method Used</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RAP 3</td>
<td>FAME</td>
<td>RTSBA6</td>
<td><em>Halomonas spp.</em></td>
</tr>
</tbody>
</table>

Checked by

Navya
(Research Analyst)
Annexure 5: Culture identification report by MTCC

Dr. G.S. Prasad
Scientist

Dr. R.D. Shelar, Associate Professor
Department of Microbiology
Zulal Bhalajino Patil College
Deopr Deul
Maharashtra-424002

July 30, 2012

Dear Dr. Shelar,

Please find enclosed herewith results of phenotypic characterization of bacterial isolate RAL6 sent by you. Based on Morphological, Biochemical and Physiological characteristics strains are showing similarity with following bacterial species. A detailed list of results is annexed for your kind information.

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Strain Designation</th>
<th>Similar to</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RAL6</td>
<td>Rheinheimera sp.</td>
</tr>
</tbody>
</table>

I thank you for your interest in MTCC.

Yours sincerely,

(G.S. Prasad)
Annexure 6: Certificate showing purity of Acetylene

Alchemie Gases & Chemicals Pvt. Ltd.

REF. NO: 1877
DATE: 08/09/2010

NAME OF CUSTOMER: M/S. S. S. SCIENTIFIC CO. DHULE- 424 001.
GAS: ACETYLENE GAS
GRADE: INSTRUMENTAL GRADE (99.6 %)
PURCHASE ORDER NO.: LETTER DT.02/09/2010
BATCH NO.: SEPT-I
CANISTER NO.: 082
WATER CAPACITY: 0.5 LTRS
FILLED PRESSURE: 17 Kg/cm²

CERTIFICATE OF QUALITY

<table>
<thead>
<tr>
<th>Acetylene</th>
<th>99.6 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impurities</td>
<td>PPM</td>
</tr>
<tr>
<td>Oxygen</td>
<td>&lt; 0.1 %</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>&lt; 0.3 %</td>
</tr>
<tr>
<td>Moisture</td>
<td>NIL</td>
</tr>
</tbody>
</table>

ANALYST

Regd. Off. & Fact. : Plot No. T - 112, M.I.D.C., Tarapur, Dist. Thane - 401 506 (Maharashtra), India. Telefax : 091-2525 - 270860, 271096 • E-mail : works@alchemiegases.com • mob. 961949032/5/7

Annexure 7: Certificate showing purity of Ethylene

<table>
<thead>
<tr>
<th>GAS</th>
<th>LEVEL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIR</td>
<td>≤ 100 PPM</td>
</tr>
<tr>
<td>ETHANE</td>
<td>≤ 400 PPM</td>
</tr>
<tr>
<td>METHANE</td>
<td>≤ 400 PPM</td>
</tr>
<tr>
<td>ACETYLENE</td>
<td>≤ 10 PPM</td>
</tr>
</tbody>
</table>