

## **APPENDIX**

## **Media and Reagents**

## Media

### 1. Nutrient Broth

gram/l

#### Quarter strength

Nutrient broth (Himedia)	2
Seawater (50%)	
pH	7.5

#### Full strength

Nutrient broth (Himedia)	8
Seawater (50%)	
pH	7.5

### 2. Amylase

Nutrient Agar(Himedia)	28
Starch	20
50% Sea water	
pH	7.5-7.8

### 3. Lipase

Peptone(Himedia)	10
CaCl <sub>2</sub>	0.1
Tween 80	10
Agar	15
50% SW	
pH	7.0-7.4

#### 4. TSB Agar

Trypticase soy broth(Himedia)	30
Granulated agar	15
Distilled water	
pH	7.5

#### 5. Mineral medium (Visscher et al. 1992)

NaCl	25
NH <sub>4</sub> Cl	0.2
CaCl <sub>2</sub> .2H <sub>2</sub> O.	0.225
KCl	0.2
MgCl <sub>2</sub> .6H <sub>2</sub> O	0.2
KH <sub>2</sub> PO <sub>4</sub>	0.02
Na <sub>2</sub> CO <sub>3</sub>	0.2
Vitamin, B <sub>12</sub>	0.002
FeSO <sub>4</sub> .7H <sub>2</sub> O	0.001
Trace element solution	1ml
HEPES Buffer	0.238
pH	7.5
Distilled Water	

##### **Trace element solution**

EDTA	0.50
ZnSO <sub>4</sub> .7H <sub>2</sub> O	0.01
MnCl <sub>2</sub> .4H <sub>2</sub> O	0.003
H <sub>3</sub> BO <sub>3</sub>	0.03
CoCl <sub>2</sub> .6H <sub>2</sub> O	0.02
CuCl <sub>2</sub> .2H <sub>2</sub> O	0.001
NiCl <sub>2</sub> .6H <sub>2</sub> O	0.002
Na <sub>2</sub> MoO <sub>4</sub>	0.003
Milli Q distilled water	

## 6. MOF test

Dextrose	10
Peptone	2
KH <sub>2</sub> PO <sub>4</sub>	0.3
Agar	15
Bromothymol blue	0.02
Seawater (50%)	

## Yeast extract

Yeast extract	300
Distilled water	30ml

## Antibiotic cocktail

Nalixidic acid	24
Piromedic acid	12
Pipemedic acid	12
NaOH saturated	150 $\mu$ L
Distilled water	30

## Stains

Gram	grams/ml
1. Crystal violet	
Crystal violet (90%)	2
Ethyl alcohol I(95%)	20
2. Gram's iodine	
Iodine	1
Potassium iodide	2
Distilled water	300
3. Safranin O	
Safranin O	2.5
Ethyl alcohol I(95%)	100

Distilled water	100
<b>Lugol's iodine solution</b>	
Iodine	10
Potassium iodide	20
Glacial acetic acid (10%)	200
<b>Acridine Orange</b>	
Acridine orange	0.1
Formalin (5%)	100

### Reagents

#### Catalase test

H<sub>2</sub>O<sub>2</sub> 3%

#### Oxidase test

N,N dimethyl paraphenylenediamine 1%

#### Buffered formalin

Formalin 38%

Hexamine

Saturate formalin with hexamine.

Filters sterilize and store at RT.

#### Protein estimation grams/ml

##### 1. Reagent A

Na<sub>2</sub>CO<sub>3</sub> 2.0

NaOH (0.1N) 100

##### 2. Reagent B

CuSO<sub>4</sub> 0.5

Sodium potassium 1.0

tartarate

Distilled water 100

3. Reagent C

Reagent A 50

Reagent B 1.0

4. Folin's Reagent (1N)

Folin's reagent (2N) 5

Distilled water 5

**FAME Analysis**

**grams/ml**

1. Reagent A - Saponification Reagent

Sodium hydroxide (certified ACS) 45

Methanol (HPLC grade) 150

Deionized distilled water 150

Combine water and methanol. Add NaOH pellets to the solution while stirring. Stir until the pellets dissolve.

2. Reagent B - Methylation Reagent

6.00N Hydrochloric Acid 325

Methanol (HPLC grade) 275

Add acid to methanol while stirring.

3. Reagent C - Extraction Solvent

Hexane (HPLC Grade) 200

Methyl tert-butyl ether (HPLC Grade) 200

Add the MTBE to the hexane and stir well.

4. Reagent D - Base Wash

Sodium hydroxide (certified ACS) 10.8

Deionized distilled water 900

Add NaOH pellets to the water while stirring. Stir until the pellets are dissolved.

**Phosphates**

1. Reagent A

Ammonium molybdate solution:

15g of reagent grade ammonium paramolybdate dissolved in 500ml deionized water.

2. Reagent B

Sulphuric acid solution: (4.5M)

250 ml of Conc. Sulphuric acid added into 750 ml deionized water.

3. Reagent C

Potassium antimonyl tartarate solution:

0.34g of potassium antimonyl tartarate in 250ml deionized water.

4. Reagent D

Ascorbic acid solution:

27g ascorbic acid in 500ml deionized water.

5. Mixed reagent:

Mix together 100ml ammonium molybdate solution, 250ml sulphuric acid solution, 50ml potassium antimonyl tartarate.

## Nitrate

1. Reagent A

N (1-naphthyl ethylenediamine dihydrochloride):

0.5g of amine in 500ml distilled water.

2. Reagent B

Sulphanilamide solution:

Dissolve 10g sulphanilamide in a mixture of 100ml conc. HCl and 500 ml distilled water. Make up the volume to 1000ml with distilled water.

3. Reagent C

Conc. Ammonium chloride solution: Dissolve 125g of AR quality ammonium chloride in 500ml of distilled water.

4. Reagent D

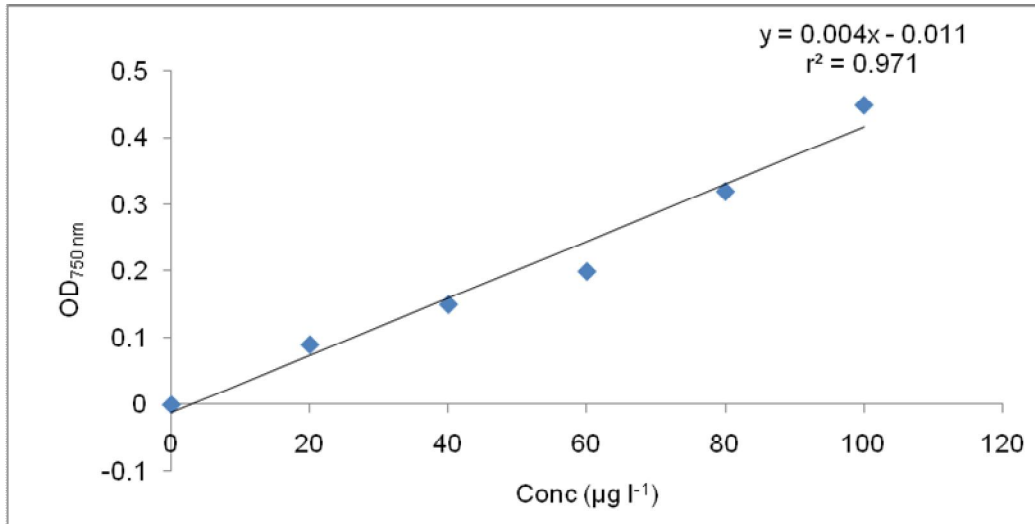


Mercuric chloride solution: Prepare 1% mercuric chloride solution by dissolving 1g of  $\text{HgCl}_2$  in 100ml distilled water.

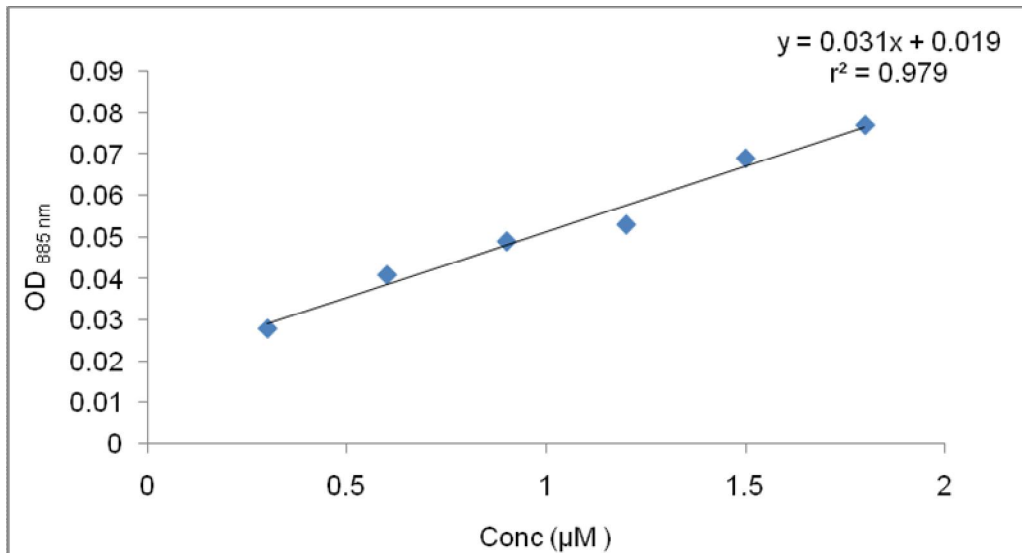
## ***Standard Graphs***

## Standard Curve

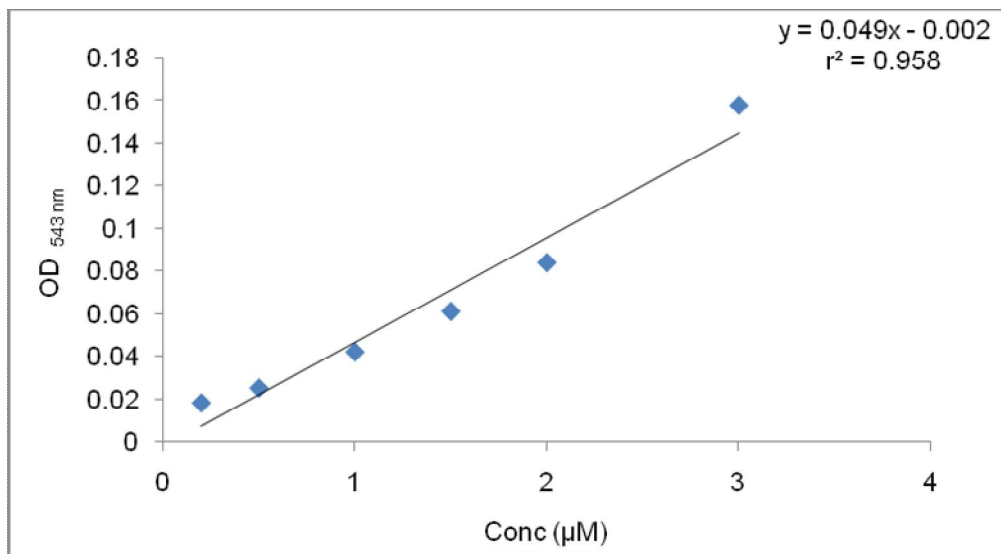
### Protein



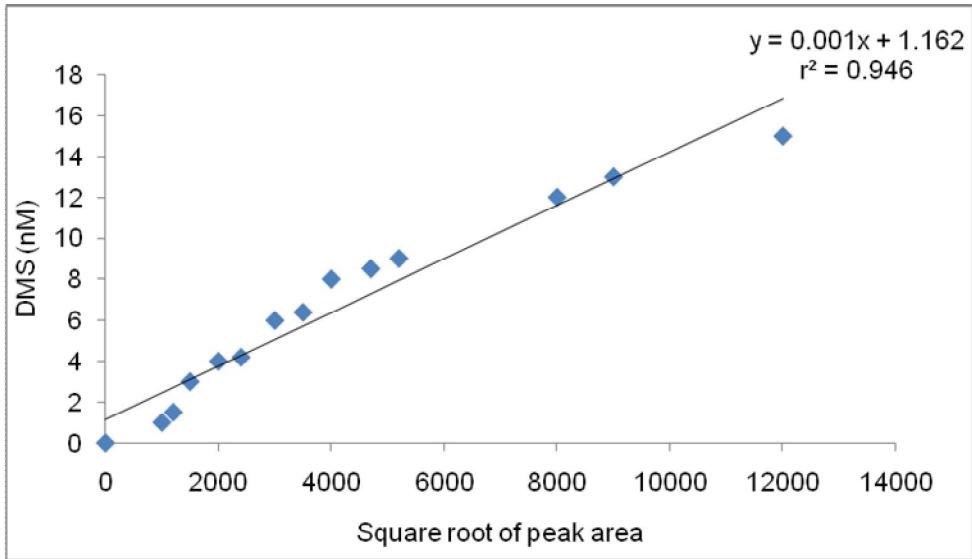
### Phosphate



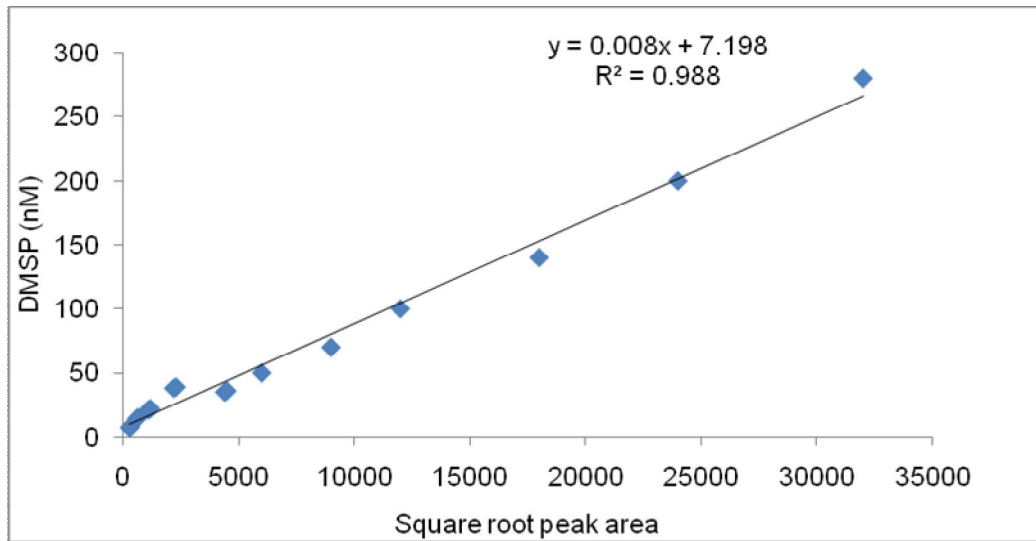
## Nitrate



## DMS



## DMSP



## **LIST OF PUBLICATIONS**

## ***Publications***

2005

- The September 2004 stench off the southern Malabar coast – A consequence of holococcolithophore bloom. Ramaiah N, Paul J.T, Fernandes V, Raveendran T, Raveendran O, Sundar D, Revichandran C, Shenoy D.M, Gauns M, Kurian S, Gerson V.J, Shoji D.T, Madhu N.V, Sree S Kumar, Loka Bharathi P.A, Shetye.S.R. *Curr. Sci.*: 88(4); 2005; 551-554.

2006

- Antiviral Property of Marine Actinomycetes Against White Spot Syndrome Virus in Penaeid Shrimps. Sree S. Kumar, Rosamma Philip, C. T. Achuthankutty. *Curr. Sci.*: 91(6); 2006; 807-811.
- Bacterial role in DMS(P) cycling. Sree S. Kumar, Usha Chinchkar, Shanta Nair, P. A. Loka Bharathi and D. Chandramohan. SOLAS News letter, III. Spring 2006. pp 8
- 4th International Symposium on Biological and Environmental Chemistry of DMS(P). Laboratory for Global Marine and Atmospheric Chemistry in the School of Environmental Sciences. 2-6 May 2006. A Meeting report. Sree S. Kumar. Challenger Wave (CSMS). August 2006

2009

- Seasonal Dimethylsulfoniopropionate (DMSP) variability in Dona Paula Bay. Sree S Kumar, Usha Chinchkar, Shanta Nair, P. A. Loka Bharathi, D. Chandramohan, *Estuarine, Coastal and Shelf Science*:81; 301-302

## ***Abstracts Presented***

*Conference of Microbiology of the Tropical Seas Oceanography (COMITS), 13 - 15 December 2004*

- Variation in DMS and its Utilization by Estuarine Bacteria. Sree S. Kumar, Chinchkar U, Nair S., P.A. Loka Bharathi, D. Chandramohan.
- Increased resistance among Penaeid shrimps to White Spot Virus when fed on diet supplemented with marine actinomycetes . Sree S. Kumar and Rosamma Philip.

*4th International Symposium on Biological & Environmental Chemistry of DMS (P) and Related Compounds. Norwich, U K 2 - 6 May 2006*

- Annual variation in Dimethyl Sulfoniopropionate (DMSP) and Dimethylsulfide (DMS) in Dona Paula Bay: Relation to biotic and abiotic factors. Sree S. Kumar, Usha Chinchkar, Shanta Nair, P. A. Loka Bharathi and D. Chandramohan.

*7th Asia Pacific Marine Biotechnology Conference. Nov 2-5, 2006.*

- Chemotactic responses in estuarine bacteria to DMSP. Sree S. Kumar, Sajan Babu Marnadiyan, Shanta Nair.