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PHYSICO-CHEMICAL ASPECTS USING ANACYSTIS NIDULANS

| T e s t s                            | Detention Period in Days |                   |                         |                         |                                 |                      |                      |
|--------------------------------------|--------------------------|-------------------|-------------------------|-------------------------|---------------------------------|----------------------|----------------------|
|                                      | Control Raw Sewage       |                   |                         |                         | Raw Sewage + Anacystis nidulans |                      |                      |
|                                      | 0                        | 2                 | 4                       | 6                       | 0                               | 2                    | 4                    |
| <u>A. P H Y S I C A L</u>            |                          |                   |                         |                         |                                 |                      |                      |
| Water temperature (°C)               | 28.0                     | 28.2              | 28.2                    | 28.2                    | 28.0                            | 28.2                 | 28.3                 |
| Colour and Turbidity                 | Brown                    | Brownish & turbid | Brownish & turbid flocs | Brownish & turbid flocs | Brown                           | Bluish green & clear | Bluish green & clear |
| pH                                   | 7.5                      | 7.8               | 7.8                     | 7.9                     | 7.5                             | 8.4                  | 9.7                  |
| <u>B. C H E M I C A L (mg/l)</u>     |                          |                   |                         |                         |                                 |                      |                      |
| Phenolphthelein alkalinity           | 34                       | 41                | 46                      | 50                      | 34                              | 72                   | 89                   |
| AM-N                                 | 28.8                     | 26.0              | 24.6                    | 22.8                    | 28.8                            | 11.9                 | 7.0                  |
| NO <sub>2</sub> -N                   | 0.20                     | 0.24              | 0.18                    | 0.22                    | 0.20                            | 0.14                 | 0.18                 |
| NO <sub>3</sub> -N                   | 0.22                     | 0.20              | 0.24                    | 0.28                    | 0.22                            | 0.20                 | 0.25                 |
| PO <sub>4</sub>                      | 18.3                     | 19.0              | 17.8                    | 16.4                    | 18.3                            | 6.8                  | 6.0                  |
| BOD <sub>5</sub> at 20°C #           | 166                      | 125               | 108                     | 58                      | 166                             | 64                   | 35                   |
| COD #                                | 280                      | 200               | 170                     | 95                      | 280                             | 98                   | 56                   |
| <u>C. B I O L O G I C A L (mg/l)</u> |                          |                   |                         |                         |                                 |                      |                      |
| Algal Biomass dry weight             | -                        | -                 | -                       | -                       | -                               | 192                  | 200                  |
| # Centrifuged Supernatant            |                          |                   |                         |                         |                                 |                      |                      |

TABLE - III

BIOLOGICAL CHANGES USING OSCILLATORIA Spp.

| Description                                                                      | Control: Raw Sewage |     |     |     | Raw Sewage + Algae |     |     |     |
|----------------------------------------------------------------------------------|---------------------|-----|-----|-----|--------------------|-----|-----|-----|
|                                                                                  | 0                   | 2   | 4   | 5   | 0                  | 2   | 4   | 6   |
| 1. Dark to light brown filament (Leptothrix Ochraceae)                           | Nil                 | C   | C   | C   | Nil                | r   | CC  | CCC |
| 2. Organic debris intermixed with the algae                                      | Nil                 | Nil | Nil | Nil | Nil                | r   | C   | CC  |
| 3. Filloculant brown precipitate separately seen at the bottom or in suspension. | Nil                 | r   | C   | CC  | Nil                | Nil | Nil | Nil |
| 4. Ciliophora (free swimming)                                                    |                     |     |     |     |                    |     |     |     |
| Paramoecium caudatum                                                             | Nil                 | r   | r   | C   | Nil                | rr  | r   | Nil |
| Paramoecium Spp.                                                                 | Nil                 | r   | rr  | Nil | Nil                | Nil | Nil | Nil |
| Aspidisca costata                                                                | Nil                 | rr  | r   | r   | Nil                | r   | C   | C   |
| Spathidium spathule                                                              | Nil                 | rr  | rrr | rrr | Nil                | Nil | Nil | Nil |
| 5. <u>Ciliophora (Stalked)</u>                                                   |                     |     |     |     |                    |     |     |     |
| Vorticella Spp.                                                                  | Nil                 | CCC | CC  | CC  | Nil                | C   | CC  | CC  |
| 6. <u>Rotifera:</u>                                                              |                     |     |     |     |                    |     |     |     |
| Lecane Spp.                                                                      | Nil                 | Nil | Nil | Nil | Nil                | r   | C   | C   |
| 7. <u>Algae:-</u>                                                                |                     |     |     |     |                    |     |     |     |
| Oscillatoria Spp.                                                                | -                   | -   | -   | -   | r                  | CC  | CC  | CC  |
| Chlorella vulgaris                                                               | -                   | -   | -   | -   | r                  | C   | C   | C   |
| Spirulina Sp.                                                                    | -                   | -   | -   | -   | r                  | r   | r   | r   |

CCC = Most Common; CC = More common

C = Common;

r = Less Common; rr = Rare

rrr = Stray form.

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TABLE - IV  
PHYSICO-CHEMICAL AND BIOCHEMICAL CONDITIONS  
USING SCENEDESMUS OBLIQUUS

| T e s t s                         | Detention Period in Days |       |       |       |       |                          |       |       |  |  |
|-----------------------------------|--------------------------|-------|-------|-------|-------|--------------------------|-------|-------|--|--|
|                                   | Control: Raw Sewage      |       |       |       |       | Raw Sewage + Scenedesmus |       |       |  |  |
|                                   | 0                        | 2     | 4     | 6     | 0     | 2                        | 4     | 6     |  |  |
| A. P H Y S I C A L                |                          |       |       |       |       |                          |       |       |  |  |
| Water Temp. (°C)                  | 29.0                     | 29.0  | 29.4  | 29.4  | 29.0  | 29.2                     | 29.2  | 29.3  |  |  |
| Colour                            | Brown                    | Brown | Brown | Brown | Brown | Green                    | Green | Green |  |  |
| pH                                | 7.7                      | 7.8   | 8.1   | 8.3   | 7.7   | 8.1                      | 8.6   | 9.0   |  |  |
| B. C H E M I C A L (mg/l)         |                          |       |       |       |       |                          |       |       |  |  |
| Amino-nitrogen                    | 32.0                     | 24.2  | 24.0  | 17.6  | 31.2  | 8.9                      | 7.6   | 5.8   |  |  |
| Phosphate                         | 14                       | 12    | 10    | 8     | 15    | 6                        | 5.4   | 4.5   |  |  |
| COD                               | 368                      | 296   | 148   | 124   | 408   | 144                      | 64    | 46    |  |  |
| C. B I O - C H E M I C A L (mg/l) |                          |       |       |       |       |                          |       |       |  |  |
| Carbohydrates                     |                          |       |       |       |       |                          |       |       |  |  |
| (a) Free Sugar                    | 41.8                     | 35.6  | 22.2  | 18.7  | 41.8  | 29.6                     | 14.8  | 15.6  |  |  |
| (b) Total Sugar                   | 110.8                    | 96.5  | 70.3  | 45.2  | 120.4 | 89.4                     | 53.3  | 20.8  |  |  |
| Protein                           | 6.1                      | 4.8   | 4.5   | 2.7   | 6.0   | 3.8                      | 3.2   | 2.0   |  |  |
| Amino-nitrogen                    | 5.1                      | 3.8   | 3.1   | 2.4   | 5.3   | 3.0                      | 2.6   | 2.1   |  |  |
| Volatile acids                    | 82.8                     | 46.8  | 30.0  | 15.6  | 84.0  | 44.8                     | 15.0  | 10.0  |  |  |
| D. B I O L O G I C A L (mg/l)     |                          |       |       |       |       |                          |       |       |  |  |
| Algal Biomass dry Wt.             | -                        | -     | -     | -     | -     | 248                      | 281   | 286   |  |  |

TABLE - V  
BIOLOGICAL CHANGES USING SCENEDESMUS OBLIQUUS

| Description                                   | Detention Period in days |     |    |    |  |                          |     |     |     |  |
|-----------------------------------------------|--------------------------|-----|----|----|--|--------------------------|-----|-----|-----|--|
|                                               | Control: Raw Sewage      |     |    |    |  | Raw Sewage + Scenedesmus |     |     |     |  |
|                                               | 0                        | 2   | 4  | 6  |  | 0                        | 2   | 4   | 6   |  |
| Dark brown filament<br>(Leptothrix Ochracea)  | nil                      | nil | r  | r  |  | nil                      | r   | c   | c   |  |
| Organic debris*                               | nil                      | r   | c  | c  |  | -                        | -   | -   | -   |  |
| Zoogloea colony<br>(Ciliophora) free swimming | rr                       | rr  | rr | r  |  | nil                      | rr  | nil | nil |  |
| Paramoecium caudatum                          | nil                      | r   | r  | r  |  | nil                      | nil | r   | rr  |  |
| Spathidium spathula                           | nil                      | r   | rr | rr |  | -                        | -   | -   | rrr |  |
| Aspidisca costata                             | nil                      | nil | rr | r  |  | nil                      | c   | c   | c   |  |
| Ciliophora (Stalked)                          | nil                      | rrr | c  | cc |  | nil                      | nil | nil | nil |  |
| Vorticella spp.                               | nil                      | rrr | c  | cc |  | nil                      | nil | nil | nil |  |

\*Organic debris is not seen separately at the bottom of the culture flasks but is seen enmeshed with the algal forms when seen under the microscope.

ccc = Most common      r = Less common  
cc = More common      rr = rare  
c = Common      rrr = Stray form

## Bacteriological examination (Sanitary Aspect) in High-Rate aerobic Oxidation

Pond using *Oscillatoria* Spp.

| Detention period<br>in days. | Control : Raw Sewage                 |                                                          | High-rate Algae treated              |                                                          |
|------------------------------|--------------------------------------|----------------------------------------------------------|--------------------------------------|----------------------------------------------------------|
|                              | Coliforms<br>(MPN/100 ml)<br>at 37°C | Total count<br>per ml at<br>37°C temp.<br>after 24 hours | Coliforms<br>(MPN/100 ml)<br>at 37°C | Total count<br>per ml at<br>37°C temp.<br>after 24 hours |
| 0                            | $16.09 \times 10^{12}$               | $10.2 \times 10^{11}$                                    | $16.09 \times 10^{10}$               | $17.9 \times 10^{10}$                                    |
| 2                            | $16.09 \times 10^{11}$               | $18.7 \times 10^{10}$                                    | $16.09 \times 10^{11}$               | $84.3 \times 10^{10}$                                    |
| 4                            | $34 \times 10^5$                     | $10.2 \times 10^7$                                       | $2 \times 10^7$                      | $68.5 \times 10^7$                                       |
| 6                            | $46 \times 10^4$                     | $14.1 \times 10^6$                                       | $18 \times 10^4$                     | $14.6 \times 10^5$                                       |



TABLE - VII

Distribution of a few important attributes among the 200 dominant bacteria isolated from the alga, Oscillatoria Spp. treated in high-rate aerobic pond on different detention periods. Fifty isolates were studied for each detention period. Results expressed in percentage of positive isolates.

| T e s t s                | Detention period in days |     |     |                          |
|--------------------------|--------------------------|-----|-----|--------------------------|
|                          | Raw Sewage<br>0          | 2   | +   | Oscillatoria Spp.<br>4 6 |
| Rods                     | 100                      | 100 | 100 | 100                      |
| Colour : Whitish         | 100                      | 100 | 100 | 100                      |
| Gram : Negative          | 100                      | 100 | 100 | 100                      |
| Flagella : Polar         | 44                       | 42  | 48  | 34                       |
| Peritrichorous           | 56                       | 58  | 52  | 66                       |
| Citrate utilizers        | 54                       | 38  | 30  | 24                       |
| Starch hydrolyzers       | 56                       | 38  | 32  | 26                       |
| Gelatin hydrolyzers      | 62                       | 66  | 0   | 0                        |
| Tributylin hydrolyzers   | 30                       | 38  | 16  | 18                       |
| Catalase activity        | 100                      | 100 | 100 | 100                      |
| <u>Reserve Materials</u> |                          |     |     |                          |
| Glycogen                 | 100                      | 100 | 100 | 100                      |
| Lipid inclusions         | 100                      | 100 | 100 | 100                      |
| Volutin                  | 100                      | 100 | 100 | 100                      |

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TABLE - VIII

Important Biochemical characteristics of the dominant  
bacteria in High-rate aerobic Oxidation Pond using  
Oscillatoria Spp. 200 Isolates studied, fifty on each day.  
Results expressed as percentage positive isolates.

| Characteristics                                                  | Detention periods in days |    |    |    |
|------------------------------------------------------------------|---------------------------|----|----|----|
|                                                                  | 0                         | 2  | 4  | 6  |
| Acid from glucose                                                | 46                        | 42 | 32 | 16 |
| Acid and gas from glucose                                        | 46                        | 30 | 32 | 26 |
| No reaction in glucose                                           | 8                         | 28 | 36 | 58 |
| Nitrate reduced                                                  | 56                        | 48 | 48 | 44 |
| H <sub>2</sub> S formed                                          | 12                        | 4  | -  | -  |
| Hugh and Leifson's $\frac{1}{2}$<br>medium glucose $\frac{1}{2}$ |                           |    |    |    |
| Oxidative                                                        | 48                        | 40 | 16 | 16 |
| Fermentative                                                     | 28                        | 24 | 26 | 26 |
| Neutral                                                          | 22                        | 26 | 40 | 40 |
| Alkaline                                                         | 2                         | 10 | 18 | 18 |

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TABLE - IX

Dominant Bacteria in High-rate Aerobic Oxidation  
Pond with Oscillatoria Spp. isolated on different  
detention periods. 200 isolates studied, fifty on  
each day. Results expressed as percentage of positive.

| Detention period in days | 0                   | 2  | 4  | 6  |
|--------------------------|---------------------|----|----|----|
| Organisms : Genera       | Percentage positive |    |    |    |
| Achromobacter            | 8                   | 4  | -  | -  |
| Aerobacter               | 8                   | 16 | 30 | 24 |
| Aeromonas                | 12                  | 4  | -  | -  |
| Alcaligenes              | 4                   | 18 | 20 | 40 |
| Azotobacter              | 2                   | -  | -  | -  |
| Bacillus                 | 12                  | 8  | -  | -  |
| Comamonas                | 2                   | -  | 16 | 18 |
| E. coli                  | 8                   | 4  | 2  | 2  |
| Proteus                  | 14                  | 6  | -  | -  |
| Pseudomonas              | 16                  | 24 | -  | -  |
| Zooglea                  | 10                  | 16 | 32 | 16 |
| Zymomonas                | 4                   | -  | -  | -  |

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TABLE - X

Dominant bacteria isolated from Assimilatory and  
Endogenous phases of a High-rate aerobic oxidation  
pond. 200 isolates studied, fifty on each day.

Results expressed as percentage of positive isolates.

| Metabolic phase              | Assimilatory |           | Endogenous |    |
|------------------------------|--------------|-----------|------------|----|
| Detention periods<br>in days | 0            | 2         | 4          | 6  |
| Organisms Genera             | Percentage   |           | positive   |    |
| Achromobacter                | 8            | -         | -          | -  |
| Aerobacter                   | 8            | 16        | 30         | 24 |
| Aeromonas                    | 12           | -         | -          | -  |
| Alcaligenes                  | 4            | 18        | 20         | 40 |
| Azotobacter                  | 2            | -         | -          | -  |
| Bacillus                     | 12           | -         | -          | -  |
| Comamonas                    | 2            | -         | 16         | 18 |
| E. coli                      | 8            | -         | -          | -  |
| Proteus                      | 14           | -         | -          | -  |
| Pseudomonas                  | 16           | 24        | -          | -  |
| Zooglea                      | 10           | <b>16</b> | 32         | 16 |
| Zymomonas                    | 4            | -         | -          | -  |

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