# INTEGRATED MICROBIAL AND ALGAL EFFLUENT TREATMENT (IMAET) BIOTECHNOLOGY FOR BIOMANAGEMENT OF LIQUID EFFLUENT

Ghatnekar, S.D.[1], Sharma, S.M.[1], Ghatnekar, S.S.[2] and Dighe, D.S.[2]
[1]Biotechnology Resource Centre, India, [2]Independent

Corresponding Author Email brc_suvash@hotmail.com

## Abstract

Present paper highlights protocol of **Integrated Microbial and Algal Effluent Treatment (IMAET)** biotechnology developed by **Biotechnology Resource Centre (BRC),** Mumbai, India. It has been successfully implemented for the bio-management of primary and secondary biodegradable liquid effluents produced by diverse industries such as gelatin, food processing, soybean oil extraction plants, paper mills, textile units etc. in India. A mathematical model to illustrate cost-benefit ratio, sustainability and environment friendly facet is described.

The liquid effluents under study yielded substantial BOD and COD loads, low pH, high TSS and TDS contents. The liquid effluents were subjected to treatment by specific enzymes and microbial consortia. Later, this pretreated effluent (50%) in combination with BRC-Spiru™ medium was utilized for cultivation of commercially important micro-algae such as *Spirulina*, *Chlorella* etc. Within 15-16 days the final treated water exhibited a significant decrease in COD by 90.08 ± 0.176%, and BOD by 89.24 ± 0.544%. The final treated discharge was applied for secondary usages. Bio-safety of the harvested biomass was tested on fish and mice.

## Keywords

Bio-safe, BRC-Spiru™ medium, effluents, enzymes, microbial consortia, gelatin, pro-biotic supplement

## Introduction

Developing countries like India have to embark upon accelerated economic development while meeting the requirements of environment preservation (Ghatnekar *et al.* 2010). Industries fulfill diverse necessities of man however, unwanted effluents are also generated. The advent of stricter environmental regulations demands a zero discharge industry. Safe disposal of these effluents projects Herculean task for the concerned industry besides creating financial burden.

Many of the present day wastewater treatment systems are ‘disposal – based linear systems’. In recent past, bio-remedial technologies for treatment of wastes are looked upon as more sustainable alternatives. Moreover, time demands a technology that could utilize these effluents to yield value added marketable products beside recovery of valuable resources mainly water and nutrients in them. It would indeed be a bonus for the industry in particular and Society in general.

Since its inception, the **Biotechnology Resource Center (BRC)**, Mumbai, India has contributed towards uniting the environment and economy by developing innovative, bio-safe waste treatment technologies of global importance (Ghatnekar and Kavian 1992; Ghatnekar *et al.* 2009b). In its advanced stride, BRC has developed a novel technology called as 'Integrated Microbial and Algal Effluent Treatment' (IMAET) biotechnology that uses microbial technology coupled with algal mass cultivation as an efficient bio-remedial measures for the treatment of secondary liquid effluents. BRC has successfully implemented IMAET biotechnology in diverse industries such as gelatine, food processing, soybean oil extraction plants, paper mills, textile units etc. in India. In this present paper a mathematical model illustrating the cost-benefit ratio, sustainability and environment friendly facet of this technology has been described.

## Categorization of Industrial wastewater

Liquid effluents generated by every industry is distinct hence needs to be characterized for its components before its successful treatment. They an be categorized as;

1. **Protein-based:** Wastewater from gelatine manufacturing plats, slaughter houses, tannery, dairy etc.
2. **Lipid-based:** Wastewater from solvent (soybean oil) extraction, edible oil refineries etc.
3. **Cellulose-based:** Wastewater from paper-mills, textile mills, sugar mills etc.
4. **Mixed effluents:** Liquid effluents are usually a combination of proteins, lipids and cellulose e.g. wastewater from antibiotics units, food processing units etc.

In general, these industrial wastewaters may contain suspended, colloidal and dissolved (mineral and organic) solids. In addition, they may be either excessively acidic or alkaline and may contain high or low concentrations of coloured matter. These wastes may contain inert, organic or toxic materials and possibly pathogenic bacteria. Unethical discharge of these improperly treated effluents can damage the public sewer system by corrosive action and silting. (Kavian and Ghatnekar, 1995). Moreover, when wastewater is decomposed biologically under anaerobic conditions develops obnoxious odour which can have undesirable impact on social health and environment.

It is crystal clear that we need to tackle two urgent problems of global proportion and magnitude food and water crisis. The researchers all over the world are constantly striving to find an alternative solution to deal with this foreseen scenario in coming decade.

## Industrial effluents – misplaced resources

Modern human civilization has witnessed rapid industrialization and urbanization. Further advancement demands successful union of environment with economy. A substantial proportion of the liquid effluents generated by industries can be considered as a valuable resource.

Over the years, BRC has devised and executed several state–of–the–art biotechnological methods for treatment of wastewater as well as bio-solids from diverse industries. These pioneer technologies involve synergistic activities of specially engineered microorganisms, enzymes, vermiculture etc. The main focus has been on conversion of bio-wastes in to coveted plant probiotics and soil conditioners which bio-safely recycled the precious mineral resources locked in the bio-wastes to plants through soil (Ghatnekar *et al.* 2009b).

## Integrated Microbial and Algal Effluent treatment (IMAET) biotechnology

The IMAET biotechnology used in present study exploits natural method of self purification and present it for the benefit of mankind. In natural water bodies and wetlands, natural forces (chemical, Physical, and solar) act to purify the water. Multiple forms of bacteria, planktons, algae and macrophytes act together or in succession, thereby achieving wastewater treatment (Ghatnekar*,* 1999; Ghatnekar and Sharma*,* 2010b). This phenomenon provides as insight upon ability of these organisms to flourish in abundant organic and inorganic matters present in the wastewater.

***Inputs for the treatment:***

1. **Wastewater**: Industrial wastewaters need to be analyzed for their physical and chemical constituents using standard methods. This is necessary to ascertain the treatment protocol, based on the characteristics of effluent i.e., its pH, salinity, BOD and COD loads, TDS and TSS contents etc.

2. **Enzymes** – Types of enzymes selected for the treatment is on the basis of effluent composition *viz.* protein, liquid or cellulose-based. Combinations of extracellular and intracellular enzymes are employed for the optimum degradation process (Table 1). Optimum combination and concentration of enzymes to yield best degradation is decided by several laboratory and pilot scale studies before commercial implementation.

**Table 1: Degrading enzymes employed depending n the types of effluents**

| Types of wastewater effluents | Degrading enzymes |
|---|---|
| Nitro/Protein-based | Proteases |
| Oil/Lipid-based | Lipases |
| Cellulose-based | Cellulases, ά-amylases, Xylases |
| Lignin-based | Lignocellulases |
| Lactose- based | Lactases |

3. **Microbial bio-cultures**- depending upon the types of effluents, a degrading bridge of microorganisms is selected. The key to the success of this technology lies in the selection of an appropriate combination of microbial consortia. General categorization of microorganisms according to their specific modus operandi is given in Table 2.

**Table 2: Categorization of microorganisms**

| Category of microorganism | Function |
|---|---|
| Deodorizers | Eliminate the abhorrent odour |
| Degraders | Decompose the complex organic components into Simple from |
| Enrichers | Enhances the quality in terms of assimilable nutrients |

BRC maintains a state-of-the-art fermentation plant at Badlapur, Maharashtra, India. This plant comprises of a rich microbial culture bank where pure cultures of various species of microorganisms including cellulose degraders, phosphate solubilizers, nitrogen fixers etc., are maintained and multiplied. BRC is actively involved in isolation, characterization and culturing of newer microorganisms from diverse source. Thereafter, their engineered isolates are prepared by acclimatizing them in specific effluents and multiplied in the fermentation plants. These isolates potentially enhance the degradation reactions.

4. **Algal cultures** – BRC maintains axenic cultures of commercially viable algae viz., *Anabaena, Spirulina, Dunaliella* etc. Algal species need to be acclimatized to a particular effluent in different stages and unique requirement of culture conditions. Natural phenomenon of succession is the most efficient method for synergistic activity of microbial and algal population in degradation of effluents.

5. **BRC Spiru™ Medium** – BRC has developed an environment friendly medium called BRC-Spiru™, for the commercial cultivation of *Spirulina* (Product literature of BRC). BRC - Spiru™ is composed of bio-safe and bio-ethical ingredients. Tamhane and Ghatnekar (2004) reported that use of BRC-Spiru™ Medium increased the growth rate of *Spirulina* culture by 40% while the time for cultivation was reduced by 27%. Moreover nutritional profile of *Spirulina* cultured in BRC- Spiru ™ Medium is better in relation to traditional chemical media (Ghatnekar and Sharma, 2010a).

6. **Treatment Tanks:** Treatment tanks consisted of a series of smooth- walled cemented and seepage- proof rectangular tanks (9 m X 7M X 1m) used for enzymatic and microbial treatment of the selected industrial wastewater. The size may vary as per the quantity of wastewater generated on daily basis.

**Figure 1: Treatment tanks for BRC's IMAET biotechnology treatment**

7. **Open channel raceway ponds:** The open channel raceway ponds (10mX3mX1.5m) are needed for mass cultivation of selected algal species using the treated wastewater from treatment tanks in combination with BRC- Spiru™ as culture medium. Motor operated paddle wheels are provided for constant agitation. Compressor with pressure control is the main aerating device. Harvesting and packing facilities are needed for the algal biomass.

**Figure 2: Open channel raceway ponds for BRC's IMAET biotechnology treatment**

***Protocol for the treatment***

The treatment protocol followed for the industrial wastewater is mentioned below.

1. **Analysis of physico–chemical characteristics of selected wastewater**

The selected wastewater is analyzed to determine the major physico-chemical parameters (pH, BOD, COD, TS, and TDS) before treatment using standard methods.

2. **Isolation and characterization of microorganisms and selection of degrading brigade**

The microbial populations in the selected wastewater are isolated and characterized.
Based on characterization, the microbial and algal species are selected and specially engineered to obtain optimum degradation results from the treatment.

3. **Acclimatization of selected microbial and algal species in wastewater**

The specially engineered effluent resistant isolates of selected microbial species are then multiplied in semi – automated fermentors (BRC-Bio-Boom).

The selected acclimatized algal strains are also multiplied in flask culture and used for further experiments. Poly-culture of selected algal species was initially maintained under specific laboratory conditions. Later, the cultures were gradually transferred to external condition.

4. **Selection of degrading enzymes**

Depending upon the characteristic study of the selected wastewater effluent, degrading enzymes are selected for the pretreatment of complex organic matter in the effluent (Ghatnekar *et.al.* 2009a).

## 5. Treatment procedure

The wastewater effluents are subjected to enzymatic and microbial treatment in series of rectangular seepage proof treatment tanks (TTs). After the saturation of microbial cultures, 70% of pretreated effluent is replaced with fresh discharge. The wastewater is monitored for the selected parameters. The total colony Forming Units (C.F.U.) and C.F.U. of selected microbial species are enumerated as indicators of biodegradation potential.

This pretreated wastewater is then mixed with BRC-Spiru™ Medium and used for culturing selected algal species in open channel raceway ponds in green house conditions. Algal cultures are regularly monitored. The optical density is used as an indicator of growth of algal species. Algal cultures attained saturation in 7-10 Days. After that the final treated discharge is pumped out and replaced with pretreated effluents from treatment tanks. The algal biomass is harvested using suitable equipments and sent for processing.

The mixed cultures of selected microbial and algal species along with degrading enzymes help in catalyzing the degradation of the organic contents in wastewater effluents. It further initiates a series of alternate aerobic and anaerobic microbial reactions resulting in exponential increase in C.F.U. count. In fine, the microorganisms and algal population exhibit excellent synergistic action in bio–treatment of selected industrial wastewater.

## Application of IMAET biotechnology: A case study of gelatine industry wastewater treatment

BRC has successfully commissioned IMAET biotechnology on commercial scale for a wide array of Indian industries including food processing, soybean oil extraction plants, pulp and paper mills, textile units, gelatine manufacturing units etc.

Recently, IMAET biotechnology is implemented for treatment of secondary wastewater effluents generated from gelatine manufacturing industry located in Vapi, Gujrat, India. During gelatine manufacturing, the plant generates 3000 $m^3$ of obnoxious wastewater every day. Conventional physical, chemical as well as biochemical treatments have failed to get rid of this obnoxious odour emanated by the effluents. This proved to be a great hurdle for that industry in meeting the strict norms laid down by the state pollution Control Board.

### *Methodology*

The waste treated was analyzed for its physical and chemical constituents (Table 4) and was further subjected to extensive studies to determine the degrading microbial brigade of selected spp. and for its standardization on Orbitek shakers.

Microbial characterization studies revealed dominance of strains of bacteria *viz., Bacillus* sp., *Cellulomonas* sp., *Nitrobacter* sp., and fungi *viz., Trichoderma* sp., *Aspergillus* sp. The selected effluents also exhibited presence of algae viz., *Oscillatoria* sp., *Anabaena* sp. etc. Presence of *Bacillus, Aspergillus* and *Trichoderma* species in gelatine waste was earlier reported by Abrusci *et al.* (2005). Based on characterization of potential degrading microbial species from the selected wastes, the following microbial and algal species were finally selected and acclimatized for the degradation experiments of selected industrial wastewater (Table 3). Cellulase and protease enzymes in ratio (2:1) were selected for the treatment considering proteinaceous nature of the effluent.

The effluents in each experimental set were monitored for the selected parameters during the treatment. The total colony – forming Units (C.F.U.) and C.F.U. of selected microbial species were enumerated as indicators of biodegradation potential. Algal cultures were regularly monitored under compound microscope. The optical density of cultures recorded using 215 D visible Spectrophotometer at 560nm.

**Table 3: Consortia of Microorganisms selected for pretreatment of gelatine effluents**

| Microorganisms | Species |
|---|---|
| **Bacteria** | *B. subtilis* (BRC-4) |
| | *N. winogradskyi* (BRC-5) |
| **Fungi** | *A. flavus* (BRC-27) |
| | *A. niger* (BRC-28) |
| **Algae** | *S. platensis* (BRC-45) |
| | *O. princeps* (BRC-46) |
| | *C. vulgaris* (BRC-47) |

***Results***

The result of physico – Chemical analysis of selected effluents after treatment exhibited significant reduction in BOD (89.24±0.544%) and COD (90.08±0.176%) values (Table 4).

The decrease in TDS content after treatment was indicative of degradation potential of the effluent. It also indicated the ability of algae to assimilate the dissolved contents as their nutrients. In present study the profound growth of algae *Spirulina* increased the alkalinity of the cultures.

**Table 4: Physico–chemical analysis of gelatine wastewater effluents before and after IMAET treatment**

| Constituents | Description | | % Reduction |
|---|---|---|---|
| | Before | After | |
| Colour | Off-white | Mild green | - |
| Odour | Abhorrent | Nil | - |
| Nature | Corrosive | Slightly slimy | - |
| pH | 6.26±0.04 | 7.02 ± 0.02 | -11.07 ± 0.307* |
| TS (%) | 0.308±0.0109 | 0.0687 ± 0.00077 | 77.56 ± 0.638 |
| TSS (%) | 0.274±0.0047 | 0.0494 ± 0.00088 | 81.97 ± 0.181 |
| BOD (ppm) | 445.00±12.45 | 47.60 ± 1.12 | 89.24 ± 0.544 |
| COD (ppm) | 1832.0±13.93 | 181.60 ± 4.43 | 90.08 ± 0.176 |

TS- Total Solids; TDS- Total Dissolved solids; BOD- Biological oxygen demand; COD- chemical oxygen demand; * -ve sign indicates rise in value

**Figure 3: Analysis of gelatine wastewater effluents before and after IMAET treatment**

## Nutritional profile of algal biomass cultured in gelatine wastewaters

The nutritional profile of algal biomass cultured in gelatine wastewater effluent (50%) and BRC-Spiru™ medium (Treated) vis-à-vis cultured in potable water with BRC-Spiru™ medium (Control) are clearly indicative that pretreated effluents did not affect the growth rate of the algal cultures; moreover the nutritional profile of cultured algae is at par with algal population cultured in potable water (Table 5).

**Table 5:** **Comparison of general composition of algal biomass cultured in selected wastewaters vis-à-vis BRC- Spiru™ medium**

| Overall compostion (% dry matter) | Control | Treated |
|---|---|---|
| Crude Protein (N x 6.25) | 60.47 | 56.84 |
| Lipids | 3.21 | 3.07 |
| Carbohydrates | 10.04 | 9.21 |
| Crude fiber | 1.57 | 1.73 |
| Ash | 7.28 | 7.0 |
| Moisture | 8-9 | 8-9 |
| **Analysis of Certain compounds (g/100g dry matter)** | | |
| Total Nitrogen | 9.78 | 9.03 |
| Non-protein nitrogen | 1.6 | 1.72 |
| Available lysine (g/100g protein) | 2.98 | 2.67 |
| RNA | 2.93 | 2.41 |
| DNA | 1.10 | 1.03 |
| Calcium | 0.72 | 0.77 |
| Phosphorus | 1.45 | 1.21 |
| Sodium | 0.45 | 0.41 |
| Magnesium | 0.92 | 0.97 |
| Iron | 0.13 | 0.13 |
| Potassium | 1.45 | 1.57 |
| **Vitamins (mg/kg dry matter)** | | |
| Thiamine | 27.8 | 24.21 |
| Riboflavin | 34.7 | 32.6 |
| Pyridoxyl-HCI | 1.3 | 1.3 |
| Cobalamine | 2.5 | 2.4 |
| Biotin | 0.07 | 0.03 |
| Beta-carotene | 500 | 430 |

**Picture 4:** ***Spirulina*** **cultured in BRC-Spiru™ medium (Control) and in gelatine wastewater (50%) and BRC-Spiru™ medium (Treated)**

## Nutraceutical trails to assess the bio-safety of the harvested algal biomass

The harvested algal biomass in gelatine industry wastewaters was tested as potential feed supplement in fish and mice. The effect of inclusion of algae on general metabolism, growth and reproductive behavior of the experimental animals was monitored.

***Trials on fish***

The nutraceutical trials were conducted on 8 carps and 42 Tilapia (polyculture system) in circular plastic collapsible pools of 1.8m diameters and 1.2m high each containing 2120-litre water. The treated feed was 65% *Spirulina* meal harvested from effluents, Groundnut oil cake 15%, and wheat bran 20% at 5% body weight. The control feed was Groundnut oil cake and 15%, and wheat bran in equal proportion. The experiment lasted for 160 days.

**Table 6: Average growth of fish fed *Spirulina* meal 65% of the diet in relation to control (poly-culture system)**

| Days | Length[cm] | | %Inc. | Breadth[cm] | | %Inc. | Weight[gm] | | %Inc. |
|---|---|---|---|---|---|---|---|---|---|
| | C | T | | C | T | | C | T | |
| 0 | 3.00 | 3.20 | 6.66 | 0.82 | 0.87 | 6.09 | 3.10 | 3.18 | 2.58 |
| 30 | 4.50 | 4.80 | 6.66 | 0.97 | 1.06 | 9.28 | 4.95 | 5.10 | 3.03 |
| 60 | 7.90 | 8.10 | 2.53 | 1.40 | 1.80 | 28.57 | 8.85 | 9.15 | 3.39 |
| 90 | 9.85 | 10.00 | 1.52 | 2.60 | 2.65 | 1.92 | 15.00 | 16.70 | 11.33 |
| 104 | 10.25 | 11.00 | 7.32 | 2.72 | 2.78 | 2.20 | 20.80 | 22.20 | 6.73 |

C-Control, T-Treated, % Inc. - Percentage increase in relation to Control.

Results indicated that *spirulina* meal at 65% level in treated had no observable adverse effects on the growth of carps and Tilapia (Table 6). Survival rate was about the same for the Treated and Control groups. Hatching occurred in 95-100 days in both control and treated pools. The number of fry was about the same in both the sets. So also the metabolic behavior that is movement and excretion was normal in both cases. Carps and Tilapias were in harmony.

Results indicated that *spirulina* meal at 65% level in treated had no observable adverse effects on the growth of carps and Tilapia (Table 6). Survival rate was about the same for the Treated and Control groups. Hatching occurred in 95-100 days in both control and treated pools. The number of fry was about the same in both the sets. So also the metabolic behaviour that is movement and excretion was normal in both cases. Carps and Tilapias were in harmony.

***Trials on mice***

In another trial, the effect of inclusion of harvested algal biomass as feed supplement in daily feed of Swiss albino mice on their growth, metabolic and reproductive behavior was also tested (Ghatnekar and Sharma 2010b). Encouragingly, it didn't exhibit any observable adverse effects on general metabolic and reproductive behaviour of the experimental animals. On the other hand increasing concentration of protein rich biomass as feed supplement enhanced the vigor of the mice. The pups of the mice also exhibited rapid growth in relation to control set where the animals were fed only standard mice feed (Table 7).

The result obtained from the nutraceutical experiment clearly indicate that the protein rich algal biomass cultured in gelatine wastewater can be effectively used as feed supplement at least in animal husbandry. In the next stage potential use of these protein supplements for human consumption is being proposed by BRC in association with universities and concerned departments from different states of India.

**Table 7: Average weight of mice administered with *Spirulina* supplement (treated groups) in relation to control**

| Parameter | Average Weight of 4 mice (gm) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Test groups | Control | | Treated | | | | | | | |
| ***Days*** | ***Robin*** | ***% Inc*** | ***Jeff*** | ***% Inc*** | ***Jim*** | ***% Inc*** | ***Bob*** | ***% Inc*** | ***Tony*** | ***% Inc*** |
| 0 | 18.72 | - | 16.81 | - | 18.01 | - | 16.87 | - | 17.08 | - |
| 15 | 23.61 | 20.71 | 19.42 | 13.43 | 24.17 | 25.48 | 22.37 | 24.58 | 23.02 | 25.8 |
| 30 | 30.0 | 37.6 | 28.75 | 41.05 | 28.62 | 37.07 | 30.0 | 43.76 | 28.75 | 40.59 |
| 45 | 32.6 | 42.5 | 30.89 | 45.58 | 30.74 | 41.4 | 30.73 | 45.10 | 31.2 | 45.25 |
| 60 | 33.3 | 43.78 | 29.75 | 43.86 | 32.5 | 44.58 | 36.3 | 53.52 | 31.25 | 45.34 |
| 75 | 34.09 | 45.08 | 34.67 | 51.5 | 33.63 | 46.44 | 38.7 | 56.40 | 37.5 | 54.45 |
| 90 | 34.8 | 46.20 | 35.2 | 52.24 | 39.0 | 53.82 | 38.2 | 55.83 | 36.0 | 52.55 |
| 105 | 36.11 | 48.15 | 35.87 | 53.13 | 39.78 | 54.72 | 40.07 | 57.89 | 39.72 | 56.99 |
| 120 | 35.02 | 46.5 | 39.04 | 56.94 | 41.03 | 56.10 | 40.39 | 58.23 | 41.3 | 58.64 |

% I Percentage increase in relation to zero day weight. ***Robin*** (Control) group fed with 100% Slandered mice feed; ***Jeff, Jim, Bob*** and ***Tony*** groups fed with 2.5g to 10g algal biomass cultured in effluents mixed with standard mice feed.


**Figure 5: Nutraceutical trials of harvested *Spirulina* on mice**

## Role of IMAET biotechnology in Clean Development Mechanism (CDM) and Carbon Credits

Since last fifteen years, BRC has successfully implemented innovative technologies for bio-management of bio-solid wastes and wastewater effluents of domestic as well as industrial origin. IMAET biotechnology involved Clean Development Mechanism (CDM). Hence the industries adopting it are likely to be rewarded with Carbon Credits. Thus IMAET biotechnology based ETPs fetches bonus to the Industry besides getting rid of effluent disposal encumbrances.

## Mathematical prototype model of IMAET biotechnology

BRC has also tested the suitability of IMAET biotechnology for the treatment of effluents from diverse industries. Based on the specific requirements of each industry BRC has generalized a low investment and cost effective mathematical prototype model of this biotechnology. The model illustrates the advantages cost-benefit ratio, sustainability, and environment friendly facet attained by an industry by the implementation of this novel biotechnology (Figure 1).

I. **Space**: The most important deciding factor for any industries undertaking this top of the line biotechnology is space requirement A typical conventional chemical based ETP set up requires 5,000-7,000 $m^2$ area whereas in 'IMAET' biotechnology based ETP, the area requirement is about half to 1/3$^{rd}$ times the space required for conventional chemical ETP.

II. **Set up:** The process involved in 'IMAET' biotechnology based ETP, are carried out in series of seepage proof tanks and algal culture ponds in green house conditions thus the infrastructure required is far simpler than in conventional ETPs.

III. **Cost:** The initial cost for commissioning this technology is just about £ 800-£ 1000 that is about 1/5$^{th}$ of the cost incurred for setting up most of other conventional ETP. Apart from maintenance of the algal cultures, no extra expenditure is required as the treatment plant is perpetual.

IV. **Conservation of Electric power and water**: Running cost of 'IMAET' biotechnology based ETP, involves marginal electricity consumption. As the energy bill is bare minimum the running costs are almost negligible. Moreover, application of this technology recovers valuable water resource that can be even recycled for in plant production processes or for secondary usages like toilets, washing and for irrigation. This recovery is itself a great conservational step.

V. **Labour:** All the phases in this treatment procedure are semi-automated and hardly 4-5 semi-skilled operators and a supervisor are required to manage a plant.

VI. **Cost effective end product**: The application of 'IMMET' biotechnology based ETP, is a lucrative venture for an industry as it offers an environment friendly zero discharge condition. Moreover the process yields commercially viable protein rich algal biomass that have tremendous market value e.g. *Spirulina platensis* has been projected as a probiotic nutraceutical food supplement, *Chlorella* already has a well established demand in the health food market several other microalgae are being researched for similar health benefits or for exploitation of secondary metabolites.

In fine, the notorious wastes of the industries can be polished into marketable nutritional food supplements for agriculture (Ghatnekar and Kavian 1992)


**Figure 1: Advantages of BRC's IMAET biotechnology treatment for industrial wastewater**

## Discussion

The waste effluents contain organic as well as inorganic components which create a high COD and BOD loads besides variable pH (NOSB TAP Review Compiled by OMRI for the USDA National Organic Program 2002). In this context, conventional treatment technologies have been further refined and new technologies for wastewater treatment are being implemented (Kumar *et al.,* 2008).

Bio-remedial measures like BRC's IMAET biotechnology for industrial wastewater treatment can be looked upon as one of the sustainable alternative to conventional waste treatment technologies. It involves synergistic action of the microorganisms and algal population in bio-treatment of industrial wastewaters. The growth of algal population in the effluents can be explained on the fact that the algae can extract nutrients from the organic matter degraded by the enzymatic and microbial treatment. In turn, the photosynthetic machinery of the algae provided aerobic environment and facilitated degradation. Kavian and Ghatnekar (1998) demonstrated the utility of fungal species viz., *A. flavus* and *A. niger* in the treatment of pharmaceutical waste.

In recent past, intensive algal cultures are being employed in outdoor ponds as a tertiary treatment for removal of residual organic compounds. As heterotrophic metabolism is faster than autotrophic, simple C sources in form of treated effluents can be employed to sustain the algal growth (Chen *et al.,* 2005). Siew- Moi (1987) suggested growth of *S. platensis* in anaerobically treated effluents from palm oil mill. Phang *et al.* (2000) reported the cultivation of *Spirulina* in anareobically pretreated effluents from sago starch factory.

## Conclusions

The Mathematical model of 'IMAET Biotechnology' suggests the appropriate solution to get rid of the diverse industrial effluents. It is advantageous over complex and high cost yet insufficient conventional effluent treatment technologies due to its features such as water resource recovery, perpetually, low cost set up and compact size. Moreover, it yields bio-safe and commercially viable protein rich biomass as profit earning product.

Nutraceutical trials of these products have proven the results on its bio-safe experimental animals and promise for human consumption after suitable trials. The least possible application of these probiotics as feed supplement in animal husbandry such as livestock, poultry, fishery, piggery etc which in turn yield important products for direct human consumption.

A successful application of this innovative biotechnology can also help industries to earn 'Carbon Credits'. The IMAET biotechnology thus has the potential to address waste management and water crisis simultaneously in addition if offers alternative to food scarcity threatening ever increasing human population globally.

## Acknowledgement

The authors would like to thank Mr. Viren Mirani (Executive Director) and Mr. D. R. Vora (C.E.O.) of I.G.C.L., Vapi for providing the required effluent samples.

## References

**Abrusci C., Martı´n-Gonza´ lez A., Del Amo A., Catalina F., Collado J. and Platas G.** (2005) Isolation and identification of bacteria and fungi from cinematographic films. Internation Biodeterioration & Biodegradation **56,** 58–68.

**Chen Y.C., Higgins M., Murthy S.N., Mass N.A., Toffey W.E. and Foster D.J.** (2005) Roles of methanogens on volatile organic sulfur compound production in anarobically digested waste water biosolids. Water Science and Technology **52(1-2),** 67-72.

**Ghatnekar S.D.** (1999) Green 2000 for industry through biotechnology- Business opportunities. Keynote address delivered in National Confereance on Industry and Environment- Challenge and Solutions in the New Millenium – CIE 99, Karad. In: Trivedy RK (Ed) Industry and Environment, Daya Publishing House, Delhi, India, pp 3-8.

**Ghatnekar S.D., Ghalsasi D.S. and Tamhane B.M.** (2009a) The novel three-tier biotechnology to convert solid waste of gelatine manufacturing unit into useful plant probiotics. *IJEP* Vol. 29 (9): 767-774.

**Ghatnekar S.D., Ghatnekar S.S. and Ghalsasi D.S.** (2009b) Three tier vermiculture biotechnology to treat bio-solid wastes into bio-clean probiotics for agriculture. 14th Europian Biosolids and organic resources conference and exhibition, Leeds, United Kingdom, organised by Aqua Enviro technology transfer (pp 1-13).

**Ghatnekar S.D., Tamhane B.M., Sawant S.A. and Sharma S.M.** (2009c) Biomanagement of liquid effluents from gelatine manufacturing industry using combination of *Spirulina platensis, Aspergillus flavus* and *Aspergillus niger. J. Indian Bot. Soc.* 88 (3-4): 165-169.

**Ghatnekar S.D., Kavian M.F., Sharma S.M., Ghatnekar S.S., Ghatnekar G.S. and Ghatnekar A.V.** (2010) Application of Vermi-filter-based effluent based treatment plant (Pilot Scale) for biomanagement of liquid effluents from the gelatine industry. *Dynamic Soil Dynamic Plant* (Special Issue 1) pp. 83-88.

**Ghatnekar S.D. and Sharma S.M.** (2010a) Effect of inclusion of *Spirulina* as probiotic feed supplement on growth of Swiss albino mice (*Mus musculus*). *Green Farming* 1(5): 535-538.

**Ghatnekar S.D. and Sharma S.M.** (2010b) Kaleidoscopic behavior of *Spirulina* under different culture conditions. In: Trivedy PC (Ed) *Plant Environment Interaction,* Pointer Publishers, 88-99.

**Kavian M.F. and Ghatnekar S.D.** (1995) Waste-water decolourisation of effluents from dye-industry using microbial cultures of twin species of *Pseudomonas.* India's 5th International Conference and Exhibition on Biotechnology. 'Biotech India 95' August 3-4, 1995, Nehru Cntre, Bombay.

**Kavian M.F. and Ghatnekar S.D.** (1998) Biotechnological innovation to treat waste from pharmaceutical industry. The Sixth Congress of Pharmaceutical Sciences of Iran., August 26-27, 1998. pp. 28.

**Kumar L., Ranjan R. and Sabumon P.C.** (2008) Development of ecologically sustainable wastewater treatment system. *Water Science and Technology* 58(1): 7-12.

**Phang S.M., Miah M.S., Yeog B.G. and Hashim M.A.** (2000) *Spirulina* cultivation in digested sago starch factory wastewater. Jpurnal of applied phycology 12(3-5): 395-400.

**Sharma S.M., Tamhane B.M., Sawant S.A. and Ghatnekar S.D.** (2008) Feeding *Spirulina* to *Mozambiq Tilapia* and Indian Major Carps. *J. Indian Bot. Soc.* 87(3-4): 248-251.

**Tamhane B.M. and Ghatnekar S.D.** (2004) Effect of chemical vis-à-vis natural ingradients in the culture media of *Spirulina platensis. In* Arvind Kumar and R. K. Somashekhar (Eds) *Environmental Science and Technology in India.* Daya Publishing House. pp. 540.