Identification of Dominant Role of Bacillus sp. in Potential Aerobic Biological Treatment of Bulk Drug Industrial Effluent

Mriganka Sekhar Mukhopadhyay, Vijay K. Dwivedi, Sudit S. Mukhopadhyay, Soumya Bhattacharyya

ABSTRACT---A year-long study has been carried out on aerobic biological treatment of bulk drug industrial effluent which is highly acidic in nature and shows high value of BOD5 (≈ 36000 mg/l), COD (≈ 84000 mg/l), and volatile solids (≈ 1, 70,000 mg/l). Chemical treatment conducted for neutralizing the pH followed by biological treatment using a lab-scale reactor with acclimatized bacterial consortia isolated from natural soil has confirmed its biological treatability. About 99% removal of COD from starting value of around 8000 mg/l has been achieved. The COD value in different hydraulic retention time (HRT) has been brought down to less than 100 mg/l in treated effluent, showing high removal of dissolved organics by aerobic biological treatment. The biochemical and genetic analysis has confirmed the Bacillus sp. playing an omnipotent role in treating the chemically treated and diluted bulk drug industrial effluent and in bringing down the COD value to a level which conform the effluent standard for discharge to surface water.

Index Terms—Keywords: Bulk Drug Effluent, Aerobic Biological Treatment, COD, Bio-kinetic Constants, Bacillus Sp.

I. INTRODUCTION

Treatment of effluent is of great concern for any kind of industry, especially for bulk drug industry. A wide variety of chemical compounds generated from bulk drug or pharmaceutical industry mix with the effluent causing highly polluted wastewater [2, 4]. It includes organic, inorganic and suspended particles which alter the pH of the water and enhance the COD i.e. Chemical Oxygen Demand and BOD i.e. Biochemical Oxygen Demand of the wastewater [26].

For the treatment of the pharmaceutical effluents, various strategies have been proposed which includes physical, chemical and biological treatment [2, 10, 12, and 26]. Biological treatment is a natural process and it plays significant role in degradation of the organic compounds [5, 17]. Both the aerobic and anaerobic biological systems have been proposed for the treatment of pharmaceutical effluents [15, 17, 27]. Anaerobic treatment is more efficient in terms of sludge yield, it deals with high concentration of wastewater, has low operation cost, causes recovery of methane gas etc. It is associated with high-capacity reactors and it requires skilled-man power for operation. Its installation cost is very high which can hardly be afforded by small bulk drug producing industries [12, 15]. Alternatively, aerobic treatment is a conventional process having low installation cost and efficient for treatment of various types of pharmaceutical wastewaters [5].

Activated sludge process is a very common aerobic treatment process which involves suspended microorganisms in the wastewater. The microorganisms are activated by air [26]. In this study acclimatized bacterial consortia isolated from natural soil has been introduced and their potentialities in bringing down the COD value to a level which conform the effluent standard for discharge to surface water has been investigated.

The objective of this present research is to develop affordable, simple wastewater treatment process for the small bulk drug producing industries. The bacterial species have been identified and the bio-kinetic constants are evaluated This will also involve identification of bacterial species and evaluation of bio-kinetic constants for understanding their potentialities in degrading the pharmaceutical effluents emanated specifically from the small bulk drug industries [1, 16, 25]. This study will definitely help the small bulk drug producing industries to treat their effluents in a most cost-effective and affordable manner by using simple reactors and the identified bacterial species responsible for biodegradation.

II. MATERIALS AND METHODS

2.1 Material

For this present research the wastewater has been collected from the equalization tank of a Kolkata based small bulk drug producing industry. For this present research all the chemicals are either AR grade or Molecular Biology grade. Double distilled water has been used for routine chemical analysis and Millipore water is used for Molecular Biology work.

2.2 Seed Preparation and Acclimatization

The inoculums for seeding the reactor have been prepared from the soil adjacent to the surface drain of the industry expecting probable presence of the specific waste degrading bacteria. The bacterial growth has been maintained in the media containing sugar, starch and peptone as carbon

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source, MgSO4, KCl, FeCl3, CaCl2, MnCl2 as micronutrients and NH4Cl along with (NH4)2HPO4 as nitrogen and phosphate source[14]. Acclimatization of the microorganisms has been done by introducing industrial effluent in the media with gradually increased dose [10, 24]. The acclimatization process has been initiated with the effluent containing the COD value of 1000 mg/lit and the concentration has gradually increased up to 8000 mg/lit with an increment of 1000 mg/lit in every stage. Each increment of 1000 mg/lit is done when the earlier dose of COD has been completely exhausted. The COD value has been measured at the interval of 6 hrs corresponding to each dose. For normal growth of the bacterium the requirement of BOD5: N: P = 100:5:1[14] has been strictly maintained. Bacterial propagation and acclimatization has been continued with constant aeration @ 2m3/hr at 300C temperature.

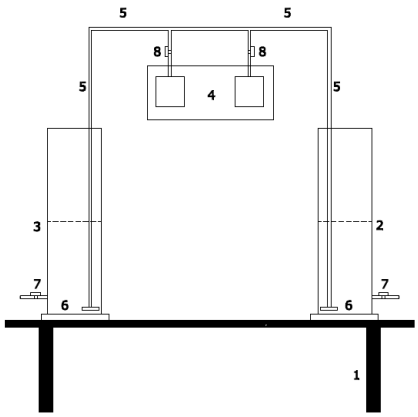

Fig.1. Schematic Diagram of the experimental setup (1- Reactor Stand, 2- Reactor I, 3- Reactor II, 4- Air pumps, 5- Aeration pipe, 6 - Air diffuser, 7- Sludge discharge way).

2.3 Reactor

A laboratory scale reactor made with cylindrical glass of 2 liter capacity has been used for biodegradation study. Constant air supply at the bottom of the reactor has been maintained by an air diffuser fitted with air pipe which is ultimately connected to an air pump. Air valve is used for controlling the volume of the air in the reactor. The reactor is equipped with an outlet facility to discharge the sludge and the wastewater (Fig 1).

2.4 Chemical Analysis

The physico-chemical characteristics {pH, COD, BOD, Total solids (TS) and Total Volatile solids (TVS)} of the wastewater have been routinely determined following the procedures provided in the Standard Methods [21].

2.5 Bacterial Identification

2.5.1. Biochemical Characterization by Staining Method

Before and after acclimatization the bacterial colonies have been microscopically identified by standard gram staining.

2.5.2 Genetic Characterization

Total genomic DNA was isolated from the soil and 16S rDNA amplified by PCR with 8F and 1492R universal primers (Fig 6-f and 6-g). The capillary sequencing was done by ABi 3730 XL DNA Analyzer machine (GCC Biotech, Kolkata)[13].

2.5.2.1 Bioinformatics Analysis

Organisms have been identified for each assay by comparing consensus sequences to a database library of known 16S rRNA gene sequences in GenBank (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) by multiple sequence alignment. The bacterial source of the sequence has been identified by matching it with a series for the maximum identity score from the GenBank database. Where more than one bacterial species have the same highest score, all species have been recorded in the results. Sequences with 97 % similarity to hits from the GenBank database have been considered to be of poor quality and has been excluded from this study. With respect to the 762/598 bp PCR, the individual 762 bp and 598 bp sequences as well as the 1445 bp consensus sequence have been analyzed from the database of GenBank and it will ensure that the two fragments have been derived from the same bacterial species.

The evolutionary relationships of 17 taxa have been deduced from UPGMA method. The optimal tree is depicted with the sum of branch length = 1.46861360. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown above the branches. The evolutionary distances have been computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. Codon positions included are 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data have been eliminated from the dataset (Complete deletion option). There are a total of 1383 positions in the final dataset. Phylogenetic analyses have been conducted in MEGA4 software.

Before and after acclimatization the bacterial colonies have been microscopically identified by standard gram staining.

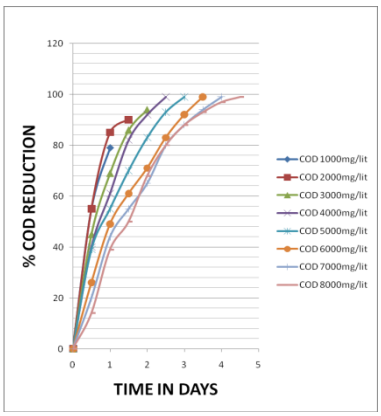

Fig.2. Acclimatization of the Microorganisms: The microorganisms have been acclimatized with the gradual increase of concentration of COD of the wastewater (1000mg/l- 8000mg/l)and their complete reduction have been studied with time. Each colour represents the concentration of COD.

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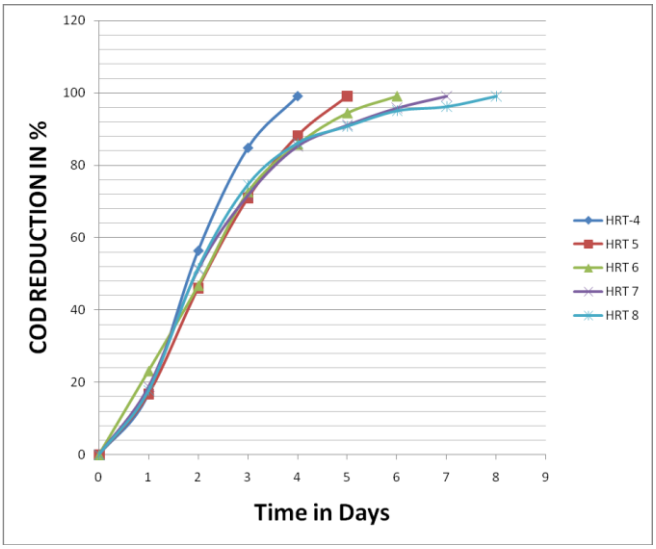

Fig.3. Percentage of COD Reduction of the effluents in the bioreactor: Several Hydraulic Retention time (HRT) has been observed (HRT-4 to 8; represents by different colour) for reduction of initial COD value (8000mg/l) to permissible level (80mg/l; approximate 100%).

III. RESULT AND DISCUSSION

3.1 Characteristics of the Wastewater

The physicochemical characteristics of the wastewater are given in the Table 1. The wastewater is highly acidic. The values of BOD and COD are found to be reasonably high due to the presence of significant amount of organic components in the wastewater sample having the value of BOD/COD = 0.44. This accounts for less biodegradability of the waste. High volatile solids content of the wastewater sample also reflects the presence of organic solids in appreciable amount.

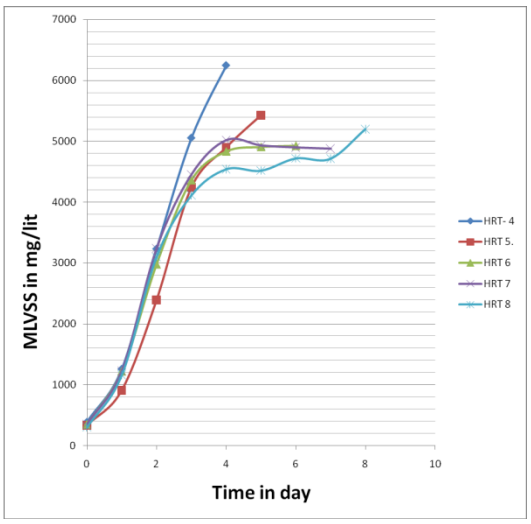

Fig.4. MLVSS Concentration in different HRT.

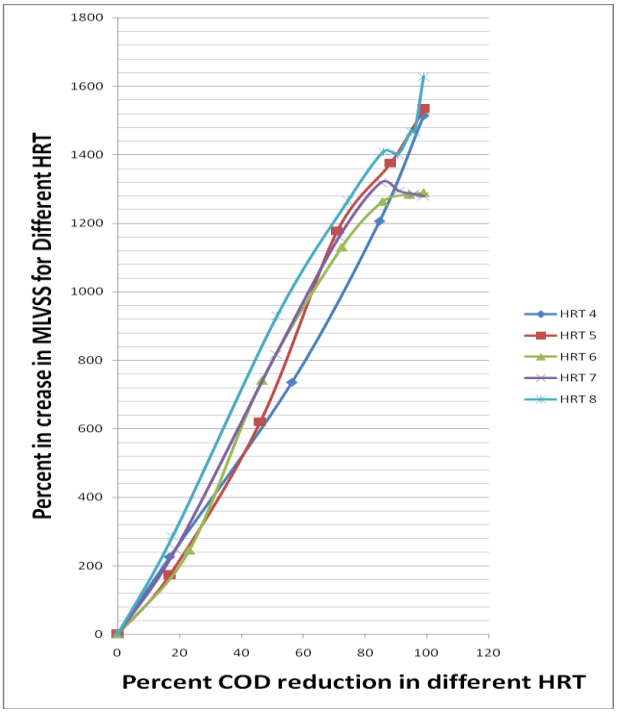

Fig.5. Correlation between percentage of increase of MLVSS concentration, vs. percentage COD reduction in different HRT.

Table 1: Characteristics of the industrial wastewater sample

Sl. No.	Parameter	Magnitude
I	pH value	3.4
II	Total solids	379.46 x 10^3 mg/l
III	Volatile solids	168.43 x 10^3 mg/l
IV	Fixed solids	188.02 x 10^3 mg/l
V	COD	83793 mg/l
VI	BOD_5	36885 mg/l

Table 2 : Characteristics of the chemically treated industrial wastewater sample

Sl. No.	Parameter	Before chemical treatment by $Ca(OH)_2$	After chemical treatment by $Ca(OH)_2$
1.	pH	3.5	7.0
2.	Total solids gm/lit	379.46	206
3.	Volatile solids gm/lit	168.43	100
4.	Fixed solids gm/lit	188.02	106
5.	COD mg/lit	83793	36008
6.	BOD_5 mg/lit	36885	30606

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Table 3: Results of COD uptake at the end of day 1 during acclimatization

Initial strength in mg/l	COD removal in %	COD removal in mg/lit
1000	79	790
2000	85	1700
3000	69	2070
4000	61	2440
5000	55	2750
6000	49	2920
7000	44	3100
8000	39	3150

3.2 Chemical Treatment

The present sample is found to have acidic characteristic, high COD value and less BOD/COD ratio. So it is not found to be suitable for biological treatment. The sample has been neutralized by $Ca(OH)_2$ and the chemical properties of the treated sample are given in Table 2. A large amount of suspended solids in the form of calcium oxalate has been generated during this process of neutralization as a result of the reaction between $Ca(OH)_2$ and oxalic acid. Solubility of Ca-Oxalate is found to be 0.67mg/100ml in cold water and most of this oxalate settles at the bottom of the container used for neutralization.

Table 4 : Analytical data of the biodegradation process

Sl. No.	Parameters	COD mg/lit	BOD_5 mg/lit
1.	Sample of untreated wastewater	83793	36885
2	Sample of wastewater after chemical treatment (pre treated)	36008	30606
3.	After bio degradation (HRT = 4days)	79.85	50.31
4.	After bio degradation (HRT = 5days)	79.09	50.02
6.	After bio degradation (HRT = 6days)	79.87	50.21
7.	After bio degradation (HRT = 7days)	81.05	51.01
8.	After bio degradation (HRT = 8days)	79.48	50.82

Table 5 : Average Biokinetic Constants Evaluated in different HRT

Sl no.	Hydraulic retention time (HRT)	Total no of Batch	K_s mg/lit	Y	K_d d^{-1}	K d^{-1}
1	HRT = 4days	7	2801	0.72	0.057	1.2
2	HRT = 5days	10	2907	0.71	0.055	1.32
3	HRT = 6 days	7	3934	0.82	0.064	1.50
4	HRT = 7days	9	2379	0.81	0.039	1.24
5	HRT = 8days	7	2634	0.35	0.051	2.17

Ca(OH)2 + HO2C-CO2H --->Ca(C2O4) + 2H2O
Calcium Oxalic acidCalcium OxalateHydroxide

The result of this experiment suggests that the chemical treatment with calcium hydroxide plays a vital role for reducing significant amount of COD value, the removal being about fifty four percent (54%). The pH remains almost neutral at 7.0 and BOD/COD ratio becomes 0.85 showing increase in biodegradability of the treated waste. Total Solids (TS) and Total Volatile Solids (TVS) are substantially reduced.

3.3 Acclimatization of the Microorganisms

The percentage COD removal corresponding to each addition of 1000 mg/lit of COD till the complete exhaustion of the same during the period of acclimatization is shown in Fig 2. It is noted from Fig 2 that uptake of COD by microorganisms is increased gradually up to the peak concentration of COD (8000 mg/lit). Table 3 shows the gradual increase in COD removal in mg/lit by microorganisms after one day for different concentrations of COD value. The increase in COD removal confirms the enhancement of biodegradation capacity of microorganisms and establishes acclimatization of the bacterial species in the environment of the bulk drug effluent.

3.4 Identification of Bacterial Organism Isolated from Soil

Routine gram staining has been performed for the identification of the bacterial organisms isolated from the soil and examined under light microscope (Fig 6- a,b,c). Microscopic examination has suggested the abundance of both the gram '+'ve and gram '—'ve bacteria and the morphological structure of the bacterium suggested the presence of both bacilli and cocci[22].

3.5 Treatability Studies of Pharmaceutical Wastes

Chemical treatment has neutralized the pharmaceutical wastewater and has also reduced the COD level in significant amount (Table 2). Further biological treatment of the wastewater has been followed in the reactor and efficiency of the bacterial organism has been evaluated with regard to the degradation of the organic material.

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3.5.1 Reduction of BOD and COD

The biodegradation in the reactor has occurred at normal climatic temperature (temperature varies in Durgapur from 120C-420C throughout the year) and the experiments have been conducted throughout year. In individual batch of experiment the COD value has been measured every day until it reaches to around 80 mg/lit and conforms the effluent standard of 250 mg/lit as per the environment protection rules of Govt. of India, 1986 [1, 7, 8, 23]. The initial COD value was 8000 mg/L and to achieve the final value of 250mg/L i.e. about 97% reduction in COD, different hydraulic retention times (HRT 4, 5, 6, 7 and 8 in days) have been observed. As the experiments have been conducted throughout the year, the variation of the climatic condition (temperature, humidity etc.) may be attributed to the change in HRT values. (Fig.-3).

Gradual increase of volatile suspended solids (MLVSS) values in the mixed liquor measured at regular interval of 1-day indicates the positive activity of the microorganisms in reducing the COD values [18]. The amount of MLVSS in different HRT has been measured and represented graphically in Fig 4. The experiment suggests that corresponding to each HRT (5, 6, 7 and 8 days) the amount of MLVSS has increased with the time and the curves have reached the steady stage. At this stage the growth rate of the microorganism has become slow due to inadequate availability of food. It is obvious that as the microorganisms are very active in degradation of chemical compounds, the COD values are reduced and MLVSS values are increased. From these two different experiments one correlation can be drawn between the percentage of COD reduction and MLVSS amount in each HRT. This correlation has given in the Fig 5, which suggests that the MLVSS amount is directly proportional to percentage of COD reduction.

The experiments have been started with the initial COD and BOD5 concentrations of wastewater around 84000 mg/l and 37000 mg/l respectively. Ultimately after chemical treatment followed by biological treatment the COD values have significantly reduced to 80 mg/l. As a consequence the BOD5 values have also been reduced to the level of 50 mg/l (Table 4). The reduction of both COD and BOD5 after biological treatment has confirmed the high efficiency of bacterial removal of organic content from the wastewater.

3.6 Evaluation of Biokinetic Constants

With starting BOD5 concentration of 5670 mg/l (the value obtained after chemical treatment and diluting the same four times with distilled water), the BOD5 values at varying θc in the reactor have been considered for evaluating biokinetic constants by using the following modified Monad's equations.

$$\frac{1}{U} = \left(\frac{K_S}{K}\right)\left(\frac{1}{S}\right) + \left(\frac{1}{K}\right) \quad (1)$$

$$U = \frac{S_0 - S}{\theta X} \quad (2)$$

$$\frac{1}{\theta_c} = YU - K_d \quad (3)$$

Where U = specific rate of utilization, mg BOD applied / mg MLVSS / day, K_s = half-velocity constant, mg/l, K = rate of utilization of substrate per day, Y = yield coefficient, K_d = decay coefficient per day, θ_c = mean cell residence time, μ_{max} = maximum specific growth rate, S = final BOD, mg/l, and S_o = initial BOD, mg/l.

The BOD based values for Ks, K,K_d and Y were 2379 – 3934 mg/l, 1.2 – 2.17 d^{-1} , 0.039 - 0.064 $d^{-1}$and 0.35 – 0.82, respectively. Average constants are cited in **Table 5** for different HRT.

3.7 Identification of Bacterial Organism Isolated from Post-Treatment Sludge

To identify the bacterial species present in the post-treatment sludge, routine gram staining has been performed and observed under the light microscope [22]. Only the gram positive bacilli have been identified (**Fig 6-d, e**). To confirm this observation Molecular Biology technique was adopted. The conserved 16S rRNA gene of the bacteria contains variable regions which are genus and species specific and characterization of the bacteria on the basis of the variable region of the 16S rRNA is a widely used method [9, 13, and 20]. The 16SrRNA gene has been amplified and further sequenced for identification of the variable region. When the sequence has been searched from the database, only the bacillus species have been identified. The sequence showed 100% homology with Bacillus strain, also it shows best homolog is *Bacillus aerius* strain 24K (ref / NR_118439.1/)[3, 6, 20] (**Fig 6-h**) by phylogenetic analysis based on 16S rRNA gene sequences.

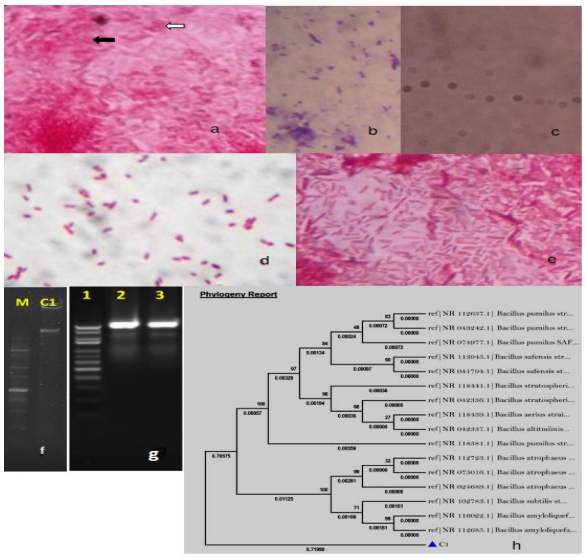

Fig.6. Identification of bacterial species: a,b and c: bacteria from pre- acclimatization soil;a: both gram positive (black arrow) and gram negative (white arrow) bacteria. b: shows bacilli and c: shows cocci forms. d and e: bacteria identified from post treatment sludge; d: gram positive and e: only bacilli form. f: lane M represents the DNA ladder and lane C1 represents the genomic DNA isolated from post treated sludge. g: Lane 1 represents the DNA ladder and lane 2 & 3 represent the PCR amplification of 16sRNA gene. h: The phylogenetic tree has drawn on the basis of the sequences of 16sRNA gene of bacteria (C1) isolated from post treated sludge.

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IV. CONCLUSIONS

Aerobic biological treatment has showed the reduction of the BOD_5 and COD of the concerned effluent to required level within short hydraulic retention time (starting from 4 days to maximum 8 days). From this study it is concluded that this treatment can be effectively carried out to treat the small-scale bulk drug industrial effluents throughout the year irrespective of change in climatic condition (temperature, humidity etc.). The most significant part of this study is identification of the dominant role of Bacillus species in treating the wastewater specifically for the small bulk drug producing industrial effluents.

REFERENCES

1. L. D. Benefield; C.W. Randall; "Activated Sludge And Its Process Modification. Biological Process Design For Wastewater Treatment"; 1980; Eagle-wood Cliffs, New Jersey, USA: Environmental Series; Prentice-Hall.
2. F. A. El-Gohary; S. I. Abou-Elela; H. I. Aly; "Evaluation of Biological Technologies For Wastewater Treatment In The Pharmaceutical Industry" 1995; Water Science & Technology, Volume 32; 13-20.
3. E. J. Feil; E. C. Holmes; D. E. Bessen; N. P. J. Man-Suen-Chan; M. C. Dayi Enright; R. Goldstein; D. W. Hood; A. Kaliai; C. E. Moore; J. Zhou and B. G. Spratt, "Recombination Within Natural Populations of Pathogenic Bacteria: Short-Term Empirical Estimates And Long-Term Phylogenetic Consequences"; 2001; Proceedings of the National Academy of Sciences of the United States of America; Volume 98; No. 1; 182-187.
4. Dos Freitas; L. M. Santos; Lo G Biundo; "Treatment of Pharmaceutical Industry Process Wastewater Using The Extractive Membrane Bioreactor" http://www3.interscience. wiley.com
5. Masters M. Gilbert; "Introduction To Environmental Engineering And Scince"; 2004; Second Edition; Prentice-Hall of India Private Limited; New Delhi-110001.
6. J. D. Gillece; J. M. Schupp; S. A. Balajee; J. Harris; T. Pearson; Y. Yan; P. Keim; E. DeBess; N. Marsden-Haug; R. Wohrle; D. M. Engelthaler; S. R. Lockhart; "Whole Genome Sequence Analysis of Cryptococcus gattii from the Pacific Northwest Reveals Unexpected Diversity" 2011; journals.plos.org/ plosone/article/journal.pone. 0028550; Volume 6; Issue 12.
7. N. F. Gray; "Wastewater treatment biological. Encyclopedia of environmental biology"; 1995; volume 3; London; Academic Press.
8. A. Hamza; "Evaluation of Treatability of the Pharmaceutical Wastewater by Biological Methods, Current Practices in Environmental Engineering"; 1984.
9. E. Jeremy Koenig; AymeSpor; Nicholas Scalfone; D. AshwanaFricker; Jesse Stombaugh; Rob Knight; T. LargusAngenent; E. Ruth Ley and R. Todd Klaenhammer; "Succession of Microbial Consortia In The Developing Infant Gut Microbiome"; 2011; Proceedings of the National Academy of Sciences of the United States of America; Volume 108; Supplement 1: Microbes and Health; 4578-4585.
10. I. Kabdasli; M. Gurel; O. Tunay; "Pollution Prevention And Waste Treatment In Chemical Synthesis Processes For Pharmaceutical Industry" 1999; Water Science and Technology; Volume 39; Number 10; 265-271(7).
11. I.B. Kasapgil; A. Selcuk; O. Ince; "Effect of Chemical Synthesis Based Pharmaceutical Wastewater on Performance, AcetoclasticMethanogenic Activity And Microbial Population In An Up Flow Anaerobic Filter"; 2002; Journal of Chem. Technol. Biotechnol.; Volume 77; 711–719.
12. S. P. Mayabhate; S. K. Gupta; and S. G. Joshi; "Biological Treatment of Pharmaceutical Wastewater" 1988; Water, Air, and Soil Pollution; Kluwer Academic Publishers; Volume 38; 189-197.
13. D. D. Medici; L. Croci; E. Delibato; S. D. Pasquale; E. Filetici; L.Toti; "Evaluation of DNA Extraction Methods for Use in Combination with SYBR Green I Real-Time PCR To Detect Salmonella enterica Serotype Enteritidis in Poultry"; 2003; Applied and Environmental Microbiology; Vol-69, No. -6, 3456 – 3461.
14. Metcalf and Eddy. "Wastewater Engineering".1995; Third Edition; New Delhi: Tata McGraw-Hill.
15. Gangagni A Rao; Venkata G Naidu; Krishna Prasad; Chandrasekhar N. K. Rao; "Anaerobic Treatment of Wastewater with High Suspended Solids from a Bulk Drug Industry Using Fixed Film Reactor (AFFR)"; 2005; Bioresource Technology; Volume 96; 87–93.
16. M. Rebhun; N. Galil; N. Narkins; "Kinetic Studies of Chemical and Biological Studies Treatment For Renovation"; 1985; Journal of Water Pollution and Control Federation; Volume 57; 324–31
17. Raj Suman; D. Samuel; Y. Anjaneyulu; "Evaluation of Biokinetic Parameters for Pharmaceutical Wastewaters Using Aerobic Oxidation Integrated with Chemical Treatment" 2005; Journal of Process Biochemistry; Volume 40, 165–175.
18. M. C. Sharma; A. K. Chaturvedi; "Simulation of biological treatment of effluents by activated sludge process at laboratory bench scale"; 1995; Indian Journal of Environ Protection; Volume 10(2); 10–14.
19. P. A. Sincero; C. A. Sincero; "Environmental Engineering - A Design Approach"; 1996; Prentice-Hall India.
20. B. Slabbinck; W. Waegeman; P. Dawyndt; P. De Vos; B. De Baets; "From Learning Taxonomies To Phylogenetic Learning: Integration of 16S rRNA Gene Data Into FAME-Based Bacterial Classification"; 2010; BMC Bioinformatics; 11:69.
21. "Standard Methods for the Examination of Water and Wastewater"; 1975; 21st edition; APHA; Washington; D.C.
22. T. Sundararaj; S. Anthoniraj; N. Kannan; S. M. Muthukaruppan; "Microbiology"; 2004; Tamil Nadu Text Book Corporation, College Road, Chennai-600 006; First Edition.
23. "THE ENVIRONMENT (PROTECTION) RULES, 1986" [The Principal rules were published in the Gazette of India vide number S.O. 844(E), dated 19.11.1986]
24. R. Vaidyanathan; "Treatability of Predigested Distillery Wastewater Diluted With Domestic Sewage". 1995; Indian Journal of Environment Protection; Volume 15(4); 241–243.
25. P. R. Vasicek; "Use of kinetic study to optimize the activated sludge process";1982; Journal of Water Pollution and Control Federation; Volume 54; 1176–1184.
26. Jakoby B. William And Bhat J. V.; "Microbial Metabolism of Oxalic Acid", 1958; www.ncbi.nlm.nih.gov/Journal List/ Bacteriol Rev; Volume 22(2).
27. YalcinAskinOktem, OrhanInce, Paul Sallis, Tom Donnelly, BaharKasapgilInce "Anaerobic Treatment of a Chemical Synthesis-Based Pharmaceutical Wastewater in a Hybrid Upflow Anaerobic Sludge Blanket Reactor" 2007; Bioresource Technology; Volume 99; 1089–1096.

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