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Dear Esteemed Readers, Authors, and Colleagues,

I hope this letter finds you in good health and high spirits. It is my distinct pleasure to address you as the Editor-in-Chief of Integrative Omics and Applied Biotechnology (IIOAB) Journal, a multidisciplinary scientific journal that has always placed a profound emphasis on nurturing the involvement of young scientists and championing the significance of an interdisciplinary approach.

At Integrative Omics and Applied Biotechnology (IIOAB) Journal, we firmly believe in the transformative power of science and innovation, and we recognize that it is the vigor and enthusiasm of young minds that often drive the most groundbreaking discoveries. We actively encourage students, early-career researchers, and scientists to submit their work and engage in meaningful discourse within the pages of our journal. We take pride in providing a platform for these emerging researchers to share their novel ideas and findings with the broader scientific community.

In today's rapidly evolving scientific landscape, it is increasingly evident that the challenges we face require a collaborative and interdisciplinary approach. The most complex problems demand a diverse set of perspectives and expertise. Integrative Omics and Applied Biotechnology (IIOAB) Journal has consistently promoted and celebrated this multidisciplinary ethos. We believe that by crossing traditional disciplinary boundaries, we can unlock new avenues for discovery, innovation, and progress. This philosophy has been at the heart of our journal's mission, and we remain dedicated to publishing research that exemplifies the power of interdisciplinary collaboration.

Our journal continues to serve as a hub for knowledge exchange, providing a platform for researchers from various fields to come together and share their insights, experiences, and research outcomes. The collaborative spirit within our community is truly inspiring, and I am immensely proud of the role that IIOAB journal plays in fostering such partnerships.

As we move forward, I encourage each and every one of you to continue supporting our mission. Whether you are a seasoned researcher, a young scientist embarking on your career, or a reader with a thirst for knowledge, your involvement in our journal is invaluable. By working together and embracing interdisciplinary perspectives, we can address the most pressing challenges facing humanity, from climate change and public health to technological advancements and social issues.

I would like to extend my gratitude to our authors, reviewers, editorial board members, and readers for their unwavering support. Your dedication is what makes IIOAB Journal the thriving scientific community it is today. Together, we will continue to explore the frontiers of knowledge and pioneer new approaches to solving the world's most complex problems.

Thank you for being a part of our journey, and for your commitment to advancing science through the pages of IIOAB Journal.

Yours sincerely,

Vasco Azevedo

Vasco Azevedo, Editor-in-Chief
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RESEARCH OPEN ACCESS

CHRONIC TOXICITY STUDIES ON PROXIMATE COMPOSITION OF *CYPRINUS CARPIO* EXPOSED TO FENTHION

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ABSTRACT

The present study indicate proximate composition including protein, carbohydrates, moisture, LSI and lipids contents of fresh water fish Cyprinus carpio chronically exposed to fenthion. In the present study the significant decrease in glycogen, protein, and increase in lipid and moisture content could be observed. Significant drop in LSI observed in Fenthion treated C. carpio clearly indicates fall in nutritional value or quality of food.

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KEY WORDS

Fenthion , Cyprinus carpio, glycogen, protein, lipid.

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[I] INTRODUCTION

All living organisms need a regular supply of energy for their survival which is obtained from surrounding sources. Food is an important source of energy used for building up the body tissue which further signifies that complete and balanced diet is necessary for the proper functioning of the body. The estimation of the rate of metabolism in a vertebrate animal has been considered as the most sensitive parameter of pollution stress since it affects many other factors such as enzyme activities, biochemical activities and other physiological processes. Keeping this view in mind it was thought to be very necessary to carry out the proximate composition study i.e. determining the proportions of carbohydrates, proteins and fats present in test tissues of the organism using standard procedure. This method helps to find out whether the insecticide, Fenthion reduces the nutritional quality of the test fish *C. carpio* which is a very good source of food. It has been shown that pesticides alter the physiological and biochemical processes in many aquatic forms [1, 11].

The information on biochemical alteration caused by Fenthion toxicity in fishes in general and on *C. carpio* in particular is not known. With this view in mind present investigation has been conducted to study the effect of sublethal concentrations of Fenthion on proximate composition which includes glycogen, protein and lipid content in the tissues of test fish. Liver somatic index changes of *C. carpio* exposed to Fenthion was also studied.

[II] MATERIALS AND METHODS

The fishes were brought from Arey fish farm in Mumbai to the laboratory. At the end of successful acclimatization healthy looking fishes of approximately the same size (13 cm.) and 18-20 gm weight were selected as test animals [12]. The fish selected for the test were exposed to three different sublethal concentrations of Fenthion. In order to maintain the concentration of toxicants throughout the period of experiment and to avoid the accumulation of metabolic wastes, entire water was replaced by fresh aged tap water every alternate day. The water analysis for determining pH, acidity, alkalinity, hardness, dissolved oxygen was carried out regularly twice a week following the standard methods [12]. The average values of all these parameters are presented in table. Healthy looking fish selected were divided into groups of eight each and were exposed to 0.38, 0.193 and 0.096 mg/l Fenthion for a period of 60 days. A control group was also maintained in duplicate for comparison. For estimation of different metabolites the control and exposed fishes were sacrificed at the end of 60 days period. The muscle and liver tissues were immediately removed for estimations of glycogen, protein, total lipids, moisture contents and Liver somatic index.

The glycogen content of liver and muscle tissues was estimated by Anthrone method [13]. The optical density was read at 625 mg on spectronic-20 (Baush & Lomb model No. 33-31-72). Lowry method [14] was opted for the determination of soluble protein contents in liver and muscle tissues. The optical density was read at 500 mg on spectronic-20. Total lipid content of dry liver and muscle tissue samples was estimated gravimetrically following the method of [15]. Moisture was determined according to the method of AOAC [16]. A known weighed quantity of muscle and liver at tissues was dried in an oven at 90°C to 95°C for 24 to 36 hours. The dried tissue was weighed again. The moisture content was calculated from the loss in weight.

$$\% \text{ Moisture} = \frac{\text{loss in wt of tissue}}{\text{wet weight of tissue}} \times 100$$

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Liver somatic index was calculated from the following formula:

$$LSI = \frac{\text{Wt. of liver}}{\text{Wt. of Body}} \times 100$$

[III] RESULTS AND DISCUSSION

The results of proximate composition of different experimental groups of fish *C. Carpio* exposed to three different sub lethal concentrations of Fenthion for period upto 60 days are given in **[Table- 1and 2]**. From comparison of the results it is evident that *C. carpio* exposed to 0.38, 0.193 and 0.096 mg/l Fenthion showed significant reduction in glycogen and protein contents of muscle and liver tissues. The LSI values decreased in the liver tissue with respect to the concentration of Fenthion exposed. Lipid and moisture contents increased with all concentrations. It can also be seen that the increasing value of lipid and moisture was proportional to the concentration of Fenthion and exposure period.

Table: 1. Changes in glycogen, protein, lipid, and moisture (mg/gm wt/wt) of muscle in *C. carpio* during cronic exposure of three different sub-lethal concentrations of Fenthion

Content	Control	096 mg/ l	0.193 mg/l	38 mg/l
Glycogen	60.63 ± 2.13	59.123 ±1.20 -2.48%	58.02 ±1.0 -4.30%	53.84* ±1.3 -11.19%
Protein	118.3 ±1.2	110.49 ±3.7 - 6.60%	109.93 ±1.6 - 7.0%	104.5 ±1.23 -11.66%
Lipid	29.8 ±1.9	31.6 ±1.0 6.04%	36.4 ±2.0 22.14%	39.1** ±2.2 31.2%
Moisture	62.93 ±1.4	64.32 ±1.5 2.20%	67.47 ±1.28 7.21%	71.44 ±1.1 13.52%

± = Standard deviation for 5 determinations in %; *= P<0.05; % =percent change from control after 60 days exposure to Fenthion. Fresh water characteristics (Average values)- Dissolved oxygen : 6.9 ±0.2; pH = 7.8 ±0.5; Carbondioxide = 0.3 ±0.2; Temperature = 29 ± 1°; Acidity in ppm = 5.6; A1ka1inity in ppm = 45.9; Total hardness (CaCO3) in ppm = 31.0

Table: 2. Changes in glycogen, protein, lipid, and moisture (mg/gm wt/wt) of liver in *C. carpio* during cronic exposure of three different sub-lethal concentrations of Fenthion

Content	Control	0.096 mg/l	0.193 mg/l	0.38 mg/l
Glycogen	12.25 ± 2.13	11.315 ±11.62 -7.63%	9.63 ±2.2 21.30%	7.64* ±1.23 -37.6%
Protein	148.3 ±12.2	140.4 ±13.7 -5.0%	134.83 ±12.6 -9.10%	129.7 ±7.23 -11.66%
Lipid	18.4 ±4.9	20.3 ±1.7 10.4%	22.5 ±7.9 22.24%	26.2* ±2.2 42.3%
Moisture	90.93 ±11.4	98.34 ±6.4 9.13%	103.92 ±11.28 15.31%	117.13 ±11.1 29.25%

± =standard deviation for 5 determinations in %; * =P < 0.05; % =Percentage change from control after 60 days exposure to Fenthion. Fresh water characteristics (Average values)- Dissolved oxygen : 6.9 ±0.2, pH = 7.8 ±0.5; Carbondioxide = 0.3 ±0.2, Temperature = 29 ± 1°; Acidity in ppm = 5.6; A1ka1 inity in ppm = 45.9; Total hardness (CaCO3) in ppm = 31.0

Glycogen represents principal and immediate source of energy. From **[Table- 1 and -2]**, it can be observed that chronic exposure of Fenthion significantly reduced the glycogen content in both the muscle and liver tissues of *C. carpio*. Ahmed et al. [17] reported reduced glycogen content in pelecypod L. marginalis exposed to Malathion. It is also reported that muscle and liver glycogen contents reduced in H. fossils when exposed to Malathion [18]. According to Dange [19], reduction in glycogen content is due to its rapid break down to release glucose into circulatory system to meet the energy requirement. This report can be supported with the increase in blood glucose level noted **[Table- 3]**, and perhaps this could also be one of the reasons for depression in glycogen content observed in *C. carpio* in the present investigation. Decrease in glycogen content observed in chronically exposed T. mossambica to Thiodan is due to tremendous increase in demand of energy [6]. Mukhopadhyay et al. [4] suggested that increased glycogenolysis decreased glycogen content in liver of Channa punctatus exposed to Malathion. It is noted that the exposure of fresh water fish *H. fossils* to concentration 0.247 mg/1 of Chlordane induced muscle and hepatic glycogenolysis and glycogenesis occurred at 2 and 12 hours exposure period [20]. According to Soman [10], glycogen content decreased with increasing glycogenolysis in C. fasciata when exposed to Lebaycid 1000. Report suggests that g1ycogen content of liver tissue of Barbus Stigma was reduced from 50 to 45 mg/g in 0.001 mg/l, 43.5 in 0.002 mg/1 and 40 in 0.003 mg/l of Endosulfan [21]. Similarly, it is observed similar reduction in glycogen contents of the liver and muscle tissues of fish Channa punctatus chronically exposed to Endosulfan [7]. Abha [22] while studying the effects of three chemicals BHC, Malathion, and RH121 on liver glycogen of fish *Trichogaster fasciatus* discussed that depletion of liver glycogen was maximum in Malathion treated followed by BHC and RH121. It is possible

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to say that in the present study, observed reduction in size of the liver of *C. carpio*, might have affected its capacity to store glycogen. Further the depletion in glycogen level noted in the present investigation could also be attributed to increased glycogenolysis to compensate the energy demand [**Tables 1-3**].

Table: 3. Changes in the biochemical compositions of blood in *C. carpio* during cronic exposure of three different sub-lethal concentrations of Fenthion

Chemical	Control	0.096 mg/l	0.193 mg/l	0.193 mg/l
Glucose mg/100 ml	62.6 ±0.8	78.8 ±0.9 25.8%	86.5 ±0.4 38.1%	98.5 ±0.2 57.3%
Protein mg/100 ml	45.9 ±0.6	44.4 ±0.2 -3.26%	40.4 ±0.24 -12.80%	33.0 ±0.35 -28.10%
Lactic acid mg/100 ml	26.4 ±0.5	27.8 ±0.3 5.30%	30.6 ±0.2 15.90%	35.6 ±0.9 34.84%
Haemoglobin gm/100 ml	10.7 ±0.1	9.95 ±0.8 -7.0%	8.6* ±0.2 -19.62%	7.7 ±0.8 -28.0%
Clotting time sec.	120.0 ±0.3	9.95 ±0.8 -7.0%	108.7 ±0.6 -9.41%	80.6** ±0.8 -32.8%

± =standard deviation for 5 determinations in %; * =P < 0.05; % =Percentage change from control after 60 days exposure to Fenthion. Fresh water characteristics (Average values)- Dissolved oxygen : 6.9 ±0.2, pH = 7.8 ±0.5; Carbondioxide = 0.3 ±0.2, Temperature = 29 ± 1°; Acidity in ppm = 5.6; A1ka1 inity in ppm = 45.9; Total hardness (CaCO3) in ppm = 31.0

From [**Table 1** and **2**] it can be revealed that protein contents in both muscle and liver tissues in chronically exposed *C. carpio* (0.38, 0.193 and 0.096 mg/l of Fenthion) decreased significantly during 60 days exposure period. The percentage of inhibition was more in liver as compared to muscle. Similar reduction in protein content was reported in *T. mossambica* exposed to Endosulfan [9]. Protein contents in muscle and liver tissues depleted in fresh water fishes exposed chronically to different insecticides [6, 10]. Rath and Mishra [23] Reported that liver exhibited maximum inhibition than muscle of *T. mossambica* when exposed to Dichlorovos. Studies noted that depletion in tissue protein in differnt species of fish exposed to various pesticides [24-26]. According to Bano [27] protein level decreased to 10% in liver of catfish exposed to Aldrin. Tissue total protein is an energy source for fishes during stress, spawning and muscular exercise [35]. Manoharan and Subbaiah [21] Observed drop in protein level from 250 mg/g to 233, 221 and 180 mg/g in 0.001, 0.002 and 0.003 mg/l respectively in Endosulfan treated Barbus Stigma. According to [28] decline in muscle and liver proteins in Seratherodon mossambica chronically exposed to DDT. Malathion, and Mercury was due to intensive tissue proteolysis. It is reported that depletion in protein content was due to histopathological changes in tissues of fresh water fish *C. fasciata* when exposed to Lebaycid chronically [10]. Studies [25, 29-31] also reveal that marked variation in activity of enzymes involved in transamination in fishes may be the cause of protein depletion. Similar changes in transaminase enzymes are observed in the present investigation Thus, in the present study decline in protein levels could be related to energy demand leading to intensive proteolysis and also due to histopathological changes [**Figures 1 (A-C)].**

A significant increase in lipid content was observed in muscle and liver tissues of Fenthion exposed fish and can be read from [**Table-1 and 2**]. Similar increase in lipid content in DDT and Dieldrin exposed *S. gairdnari* and in *T. mossambica* on exposure to Thiodan are also reported [6, 31]. Increase in lipid content in *C. fasciata* when exposed to insecticide Lebaycid is also noticed [10] and increase in lipid content of fresh water fishes exposed to Endrin and Lebaycid, respectively are also reported [3, 33]. According to Blazka [34] lipids are formed as the end product of carbohydrate metabolism especially in anerobic and sluggish fish. It is suggested that tissue hypoxia might have played significant role in synthesis of lipid for carbohydrate precursors in fish exposed to DDT [32]. In the present study the significant increase in lipid content could be due to tissue hypoxia and it could also be attributed to fall in glycogen and protein contents which in turn are compensated with rise in lipid content so as to withstand the stress of toxicant.

The increase in the moisture content of the muscle and liver of the fish exposed to all the concentrations 0.38, 0.193, 0.096 mg/1 of Fenthion was maintained till the end of 60 days experimental period [**Tables 1 and 2**]. Love Malcolm [35] postulated that the fish may at first consume lipid from the liver and start to mobilize muscle proteins, only after this source of energy depletes subsequent to the utilization of muscle proteins, water moves in to take its place and thus resulting in the increase in moisture content. The increased levels in moisture content were [6] in *Tilapia mossambica* exposed to Thiodan and PMA. Soman [10] also noted similar observation in *C. fasciata* exposed to Lebaycid 1000. Hence, in the present study the cause for rise in moisture content could be due to the subsequent utilization of muscle proteins.

In the present investigation a significant drop in LSI was observed in Fenthion exposed *C. carpio* [**Table 4**]. According to Gaikwad [6] low LSI value in T. mossambica exposed to Thiodan Could be due to the damage caused to liver by the pesticide or by the pollutant. Rath and Mishra [23] studied change in LSI value in the liver and reported that LSI value

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reduced when T. mossambica was exposed to Dichlorovos and further discussed that it could be due to loss of cells of respective tissue. Hence, in the present study taking the support of above workers, the reduction in somatic index of liver of exposed fish *C. carpio*, could be attributed to the loss of somatic cells, bio- chemical changes and may also be due to histopathological lesion of liver cells [**Figure-1 (A-D)**].

Table: 4. Changes in liver somatic index (LSI) in *C. carpio* during cronic exposure of three different sub-lethal concentrations of Fenthion

Content	Control	0.096 mg/l	0.193 mg/l	0.38 mg/l
Liver Somatic Index (LSI)	1.93 ±0.05	1.70 ±0.03	1.15 ±0.03	0.89 ±0.01

± =standard deviation for 5 determinations in %; * =P < 0.05; % =Percentage change from control after 60 days exposure to Fenthion.

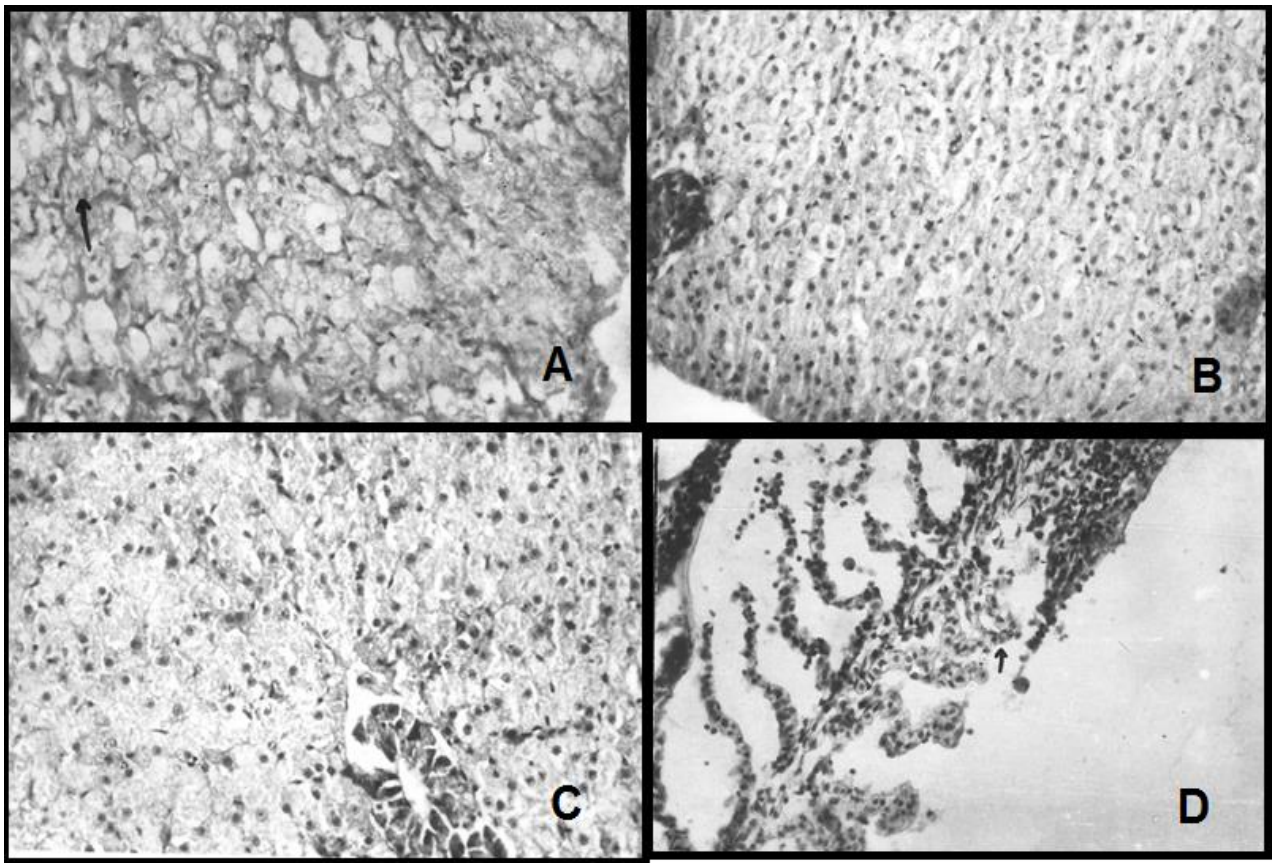

Fig:1. A) Liver of *C. carpio* exposed to 0.38 mg/ml fenthion showing vacuolated, cloudy swollen, disintegrated and extremely ruptured hepatic cells. B) Liver of *C. carpio* exposed to 0.096 mg/ml showing pycnosis and large number of necrotic regions. C) Liver of *C. carpio* exposed to 0.096 mg/ml showing large no of fatty degeneration and disturbed cordal arrangement of hepatocytes. D) Gill of *C. carpio* exposed to 0.38 mg/ml.Fenthion for 60 days exposure showing vacuolated, deformed and shortened secondary lamellae (arrow mark).

[VI] CONCLUSION

Reduction in protein and carbohydrate contents of Fenthion treated *C. carpio* indicates fall in nutritional value or quality of food. The increase in moisture content may possibly be as a result of the change occurred in the energy sources while the increase in lipid content indicates the alternative mechanism induced to compensate the toxicity stress. Decrease in LSI value probably denotes the damage caused to liver cells in response to stress induced by Fenthion.

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CONFLICT OF INTEREST

Author declares no conflict of interest.

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NIL

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REVIEW ARTICLE OPEN ACCESS

DEGRADATION OF PULP AND PAPER MILL EFFLUENTS

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ABSTRACT

Pulp and paper mills are categorized as a core sector industry and are the fifth largest contributor to industrial water pollution. Pulp and paper mills generate varieties of pollutants depending upon the type of the pulping process. Pulp and paper mill effluents pollute water, air and soil, causing a major threat to the environment. Although the physical and chemical methods are on the track of treatment, they are not on par with biological treatment because of cost ineffectiveness and residual effects. The biological treatment is known to be effective in reducing the organic load and toxic effects of kraft mill effluents. None of the available conventional methods are permanent eco-friendly disposal solution. Biological methods have been acknowledged for the degradation of pulp and paper mill effluents. Biological methods involve microorganisms capable of degrading pulp and paper waste in natural environments. The biological colour removal process uses several classes of microorganisms- bacteria, algae and fungi-to degrade the polymeric lignin derived chromophoric material. Several methods have been attempted by various researchers throughout the world for the removal of colour from pulp and paper mill effluents.

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KEY WORDS

Pulp, paper, effluents, microorganism

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[I] INTRODUCTION

Pulp and paper mill is a major industrial sector utilizing a huge amount of lignocellulogic materials and water during the manufacturing process, and release chlorinated lignosulphonic acids, chlorinated resin acids, chlorinated phenols and chlorinated hydrocarbon in the effluent [1]. The highly toxic and recalcitrant compounds, dibenzo-p-dioxin and dibenzofuran, are formed unintentionally in the effluent of pulp and paper mill [2, 3]. The untreated effluents from pulp and paper mills discharged into water bodies, damages the water quality. The undiluted effluents are toxic to aquatic organisms and exhibit a strong mutagenic effect. Several physical, chemical and biological methods are used for the removal of colour from the pulp and paper mill effluents. Physical and chemical processes are quite expensive and remove high molecular weight chlorinated lignins, colour, toxicants, suspended solids and chemical oxygen demand. But BOD and low molecular weight compound are not removed efficiently [4]. The biological colour removal process is particularly attractive since in addition to colour and COD it also reduces BOD and low molecular weight chlorolignins [5, 6]. Microorganisms rapidly degrade a few chemicals and eliminate them from the environment, but there are other chemicals that are degraded slowly, accumulate in the environment and occasionally exhibit toxicity [7]. Biodegradation of hazardous harmful substances in the environment embody significant prospective methods, when complex and ecologically unsound pollutants are decomposed into simpler substances (sound ones) by the action of microorganisms. The principle of biodegradation technologies is an optimization of nutrient ratios (to support the growth of selected microorganisms able to degrade the target contaminants) and an application of suitably selected isolated microorganism strains with relevant degradation abilities [8]. Treatment of pulp and paper mill effluent has not proved successful due to lack of suitable microorganism, loss of genetic potentiality in adverse environmental conditions, formation of recalcitrant compounds of various structural formulation and poor process optimization for treatment at large scale. Although the physical and chemical methods are on the track of treatment, they are not on par with biological treatment because of cost ineffectiveness and residual effects. The biological treatment is known to be effective in reducing the organic load and toxic effects of krafts mill effluents [9]. The microorganism treats the effluents mainly by two process; action of enzymes and biosorption [10]. The various enzymes involved in the treatment of pulp and paper mill effluent are lignin peroxidase, manganese peroxidase and laccase [11]. Microorganisms showing good production of these enzymes have the potency to treat the effluent.

[II] PULP AND PAPER MILL

The manufacture of papers dates to the ancient Egyptians before 3000 B.C., while the 'modern' method of pulping plant material for paper production was developed by the Chinese in the first century A.D. The utilization of plant fiber for paper production is one of the oldest manufacturing industries and is built upon age-old technologies. It was not until this became mechanized and the scale of production escalated in the early part of last

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century that many of today's environmental problems associated with the pulp and paper industry emerged. For example, in the industrial manufacture of paper from wood fiber, it was known that natural compounds released during processing caused harm to aquatic population [12]. Pulp and paper are manufactured from raw materials containing cellulose fibers, generally wood, recycled paper, and agricultural residues. In developing countries, about 60% of cellulose fibers originate from nonwood raw materials such as bagasse (sugar cane fibers), cereal straw, bamboo, reeds, esparto grass, jute, flax, and sisal. In World Bank studies [13], pulp and paper manufacturing with unit production capacities greater than 100 metric tons per day. As per the Ministry of Environment and Forest (MoEF), Government of India, the pulp and paper sector is in the "Red Category" list of 17 industries having a high polluting potential. Pulp and paper production is a major industry in India with a total capacity of over 3 million tons per annum [14].

[III] CHARACTERISTICS OF PULP AND PAPER MILL EFFLUENTS

The pulp and paper industry produces effluents with large BODs and CODs. One of the specific problems that yet not been solved is the strong black brown color of the effluent, which is primarily due to lignin and its derivatives released from the substrate and discharged in the effluents, mainly from pulping, bleaching and chemical recovery stages. The brown color of the effluent may increase water temperature and decrease photosynthesis, both of which may lead to decreased concentration of dissolved oxygen [15].

The generation of waste water and characteristics of pulp and paper mill effluent depends upon the type of manufacturing process adopted and the extent of reuse of water employed in plant. Effluent depends upon type of manufacturing process adopted and the extent of reuse of water employed in plant. Effluent of kraft pulping is highly polluted, and characterized by parameters unique to these wastes such as colour, adsorbable organic halides (AOX) and related organic compound. The alkaline extraction stage of bleach plant effluent is the major source of colour and is mainly due to lignin and derivatives of lignin [16]. Lignin wastewater is discharged from the pulping, bleaching and chemical recovery sections. Lignin is a heterogeneous, three dimensional polymer, composed of oxyphenylpropanoid units. The high chlorine content of bleached plant reacts with lignin and its derivatives formed into highly toxic and recalcitrant compounds and the responsible for high biological and chemical oxygen demand. Trichlorophenol, trichloroguicol, tetrachloroguicol, dichlorophenol , dichoroguicol and pentachlorophenol are major contaminats formed in the effluent of pulp and paper mill [17]. The pollutants at various stages of the pulping and paper making process are presented in **Figure- 1**[18].

Owing to its serious pollution threat, it is mandatory for pulp and paper mills to take appropriate measures to comply with the discharge standards set by the Central Pollution Control Board (CPCB) [19], which is the national agency responsible for environmental compliance. The minimum national standards for pulp and paper mills wastewater discharge according to CBCP are shown in **Table-1.**

Table: 1. Minimum national Standards for pulp and paper mills wastewater discharge (CPCB, 2000)

PARAMETER	LARGE PAPER MILLS	SMALL PAPER MILLS
PH	6.5-8.5	5.5-9.0
SUSPENDED SOLIDS (MG/L)	100	100
BOD AT 27^0C(MG/L)	30	INLAND: 30 Land: 100
COD (MG/L)	350	-
TOTAL ORGANIC CHLORINE (TOCL) **(kg/ton paper 1992 onwards)**	2.0	-
SODIUM ABSORPTION RATIO (SAR)	-	26

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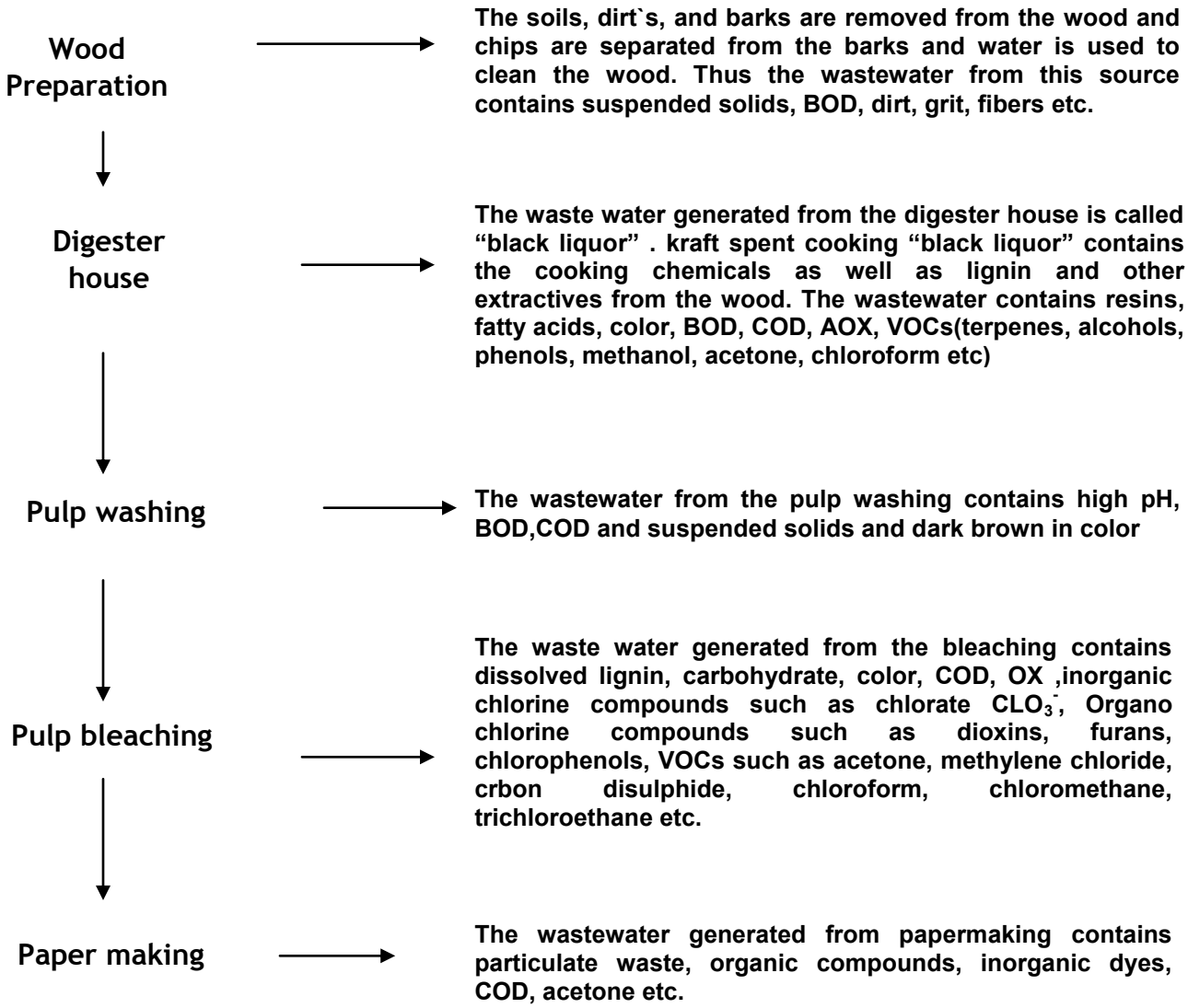

Fig: 1. Pollutants from various sources of pulping and papermaking (US EPA, 1995)

[IV] TECHNOLOGIES USED FOR THE TREATMENT OF PULP AND PAPER MILL EFFLUENTS

Recent developments in treatment of pulp and paper mill wastewater showed successful application of physical, chemical and biological treatment methods as well as combination of different methods in series. Commonly used physical and chemical treatment methods are electrocoagulation [20], ultrasound [21], reverse osmosis [22], photocatalytic systems using titanium dioxide (TiO_2) and zinc oxide (ZnO) under UV/solar irradiation [23], hydrogen peroxide, Fenton's reagent (H_2O_2/Fe^{2+}), UV,UV/ H_2O_2, photo-Fenton (UV/ H_2O_2/Fe^{2+}), ozonation and peroxon (ozone/ H_2O_2)[24]. Some of these studies have optimized the operating conditions for effluent treatment [24-26]. Biological treatment methods involved the use of fungi, bacteria, algae and enzymes [27] as a single step treatment or in combination with other physical and chemical methods [28-30]. The biological treatment studies have confined themselves to the evaluation of microorganism, basic mechanism behind treatment and changes in the effluent after treatment. Not even a single study has optimized the process of effluent treatment. The microorganism treats the effluent mainly by two process: action of enzymes and biosorption as shown in **Figure–3** [31]. The various enzymes involved in the treatment of pulp and paper mill effluent are lignin peroxidase, manganese peroxidase, and laccase [11]. Microorganism showing good

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production of these enzymes have the potency to treat effluent. Biological treatment systems are particularly attractive, since in addition to colour they also reduce the BOD and COD of the effluent [27].

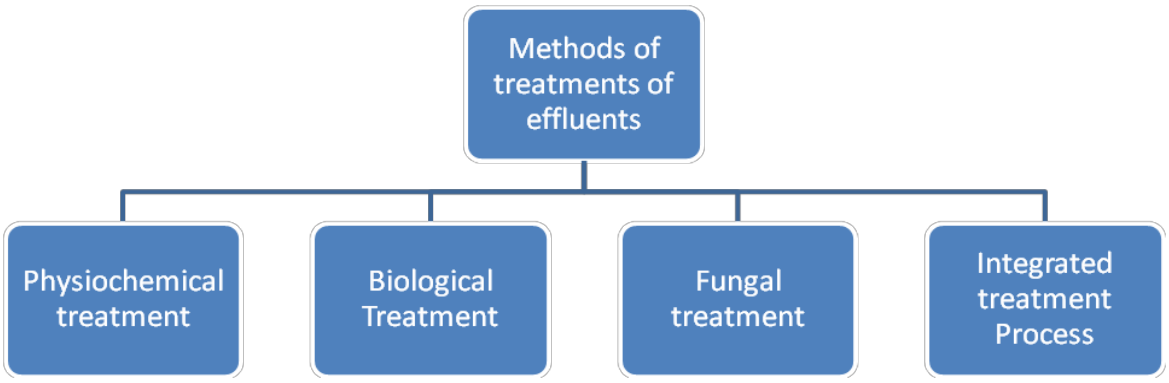

Fig:2. Figure showing different techniques used in the treatment of pulp and paper mill effluents

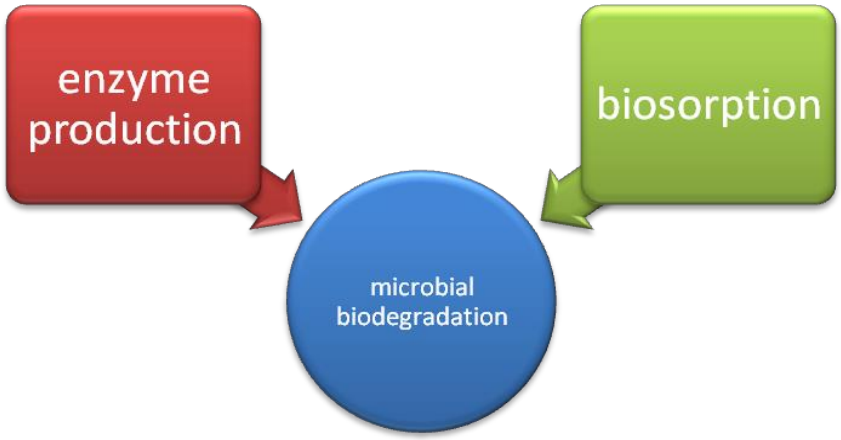

Fig: 3. Figure showing the two main process of microbial biodegradation of effluents

[V] ENVIRONMENTAL IMPACT OF PAPER AND PULP MILLS

The environmental impact of paper and pulp mills is of particular concern since these units generate 150-200 m^3 effluent/ton paper with a high pollution loading of 90-240 kg suspended solids /ton paper,85-370 kg biochemical oxygen demand (BOD)/ton paper and 500-1100 kg chemical oxygen demand (COD)/ton paper [32]. Apart from the pollution, there is a growing water scarcity and deterioration in water quality in many parts of India [33]. Thus, in the context of reduced freshwater availability, declining water quality and environment pollution from inadequately treated effluent, there is an urgent need for efficient water management in pulp and paper mills. About 500 different chlorinated organic compounds have been identified in paper mill effluents [34]. The high chemical diversity of these pollutants causes a variety of clastogenic , carcinogenic, endocrinic and mutagenic effects on fishes and other aquatic communities in recipient water bodies [35-36].

[VI] FATE AND AFFECTS OF PULP AND PAPER MILL EFFLUENTS

Various studies have reported detrimental effects of pulp and paper mill effluent on animals living in water bodies receiving the effluent. The effects are in form of respiratory stress, oxidative stress, liver damage and geno-toxicity [37-39]. A study in 1996 reported health impacts such as diarrhea, vomiting, headaches, nausea, and eye irritation on children and workers due to the pulp and paper mill wasterwater discharge to the environment [40]. The effluent has high chemical diversity of organic chemicals present in it. Many of them are carcinogenic, mutagenic, clastogenic and endocrinic disrupters. A study on *B.subtilis* reported the mutagenic effects of the sediments contaminated by the effluent of kraft paper mill [41].

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Another study reports the toxic and mutagenic effects of pulp and paper mill effluent contaminating lake Baikal [42]. Exposure to the effluent adversely affects diversity and abundance of phytoplankton, zooplankton and zoobenthos, disrupting benthic algal and invertebrate communities [36]. Therefore it is obligatory to treat the effluent before disposal.

[VII] NEED TO SEARCH A NEW TECHNOLOGY

In recent past, the colour of effluent discharge into waterways has become important problem. Pulp paper mill effluent has recognized as environmental hazards and categorized one of the twelve most polluting industry in our country. The dark brown colour of the effluent is mainly due to their high contents of oxidized and partially degraded lignin. Reducing this colour before the effluent is mainly due to their high contents of oxidized and partially degraded lignin. Reducing this colour before the effluents are discharged into natural water is an important goal. Other toxic contaminants of pulp and paper mill industry are chlorinated compounds [43, 27]. Physical and chemical methods undertaken to study colour removal from the effluent is not found to be cost-effective technology. Hence, biological treatment has been applied for the decolourization of effluent of pulp and paper mills. An important strategy for effluent treatment is the isolation and characterization of genetically significant microorganisms together with designing and optimization of process parameter to deal with specific environment pollutants [44].

[VIII] ROLE OF MICROBES IN THE DEGRADATION OF PAPER MILL EFFLUENT

Microbial biodegradation is carried out by different organisms like Bacteria, Fungus, and Algae [45-46]. Effective Microorganism (EM) is the consortia of valuable and naturally occurring microorganisms which secretes organic acids and enzymes for utilization and degradation of anthropogenic compounds [47]. These days, microbes are collected from the waste water, residual sites and distillery sludges which are believed to have the resistance against the hazardous compounds. This is particularly due to their tolerance capacity even at the higher concentrations of xenobionts [48]. Bioremediation process involves detoxification and mineralization, where the waste is converted into inorganic compounds such as carbon dioxide, water and methane [49]. When compounds are persistent in the environment, their biodegradation often proceeds through multiple steps utilizing different enzyme systems or different microbial populations [50, 51].

[IX] CONCLUSION

This review article may therefore serve as a challenge to researchers to continue developing better methods to degrade the effluents. Although decolourization is a challenging process to the waste water treatment of pulp and paper mill, the result of this findings and literature suggest a great potential for microorganism to be used to remove color from wastewaters. The microorganisms are adaptive in nature and can degrade contaminants. The ability of the strain to tolerate, decolorize the toxic effluents at high concentration gives it an advantage for treatment of textile industry waste waters. However, potential of the strain needs to be demonstrated for its application in treatment of effluents. Environmental problems caused by the industrial effluents are mainly due to accumulation of pollutants having toxic compounds. There is a quick need to degrade these toxic compounds in an eco-friendly way. Microbial degradation technique has no negative impact on the environment. They degrade the toxic compounds in their own ways. Various techniques like microbial remediation, phytoremediation and photoremediation and their subtypes have been used as a eco-friendly methods. Although slow, on the whole microbial bioremediation was found to cover wide range of recalcitrant degradation and is known to be a better choice because of its nature of degradation.

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CONFLICT OF INTEREST
Author declares no conflict of interest.

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RESEARCH ARTICLE OPEN ACCESS

PROTEIN MODELLING AND IDENTIFICATION OF BINDING INTERACTIONS BETWEEN FAT10 AND MAD2

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ABSTRACT

Chromosome instability involving gains and loss of chromosomes has been found to occur in most malignancies. Genes responsible for chromosomal instability in human cancers include mitotic "check point" genes that moniter the proper progression through the cell cycle. MAD2 is a key mitotic spindle checkpoint protein whose primary role is to ensure that all of the chromosomes are properly attached to the mitotic spindle before the onset of anaphase. FAT10, a member of the ubiquitin-like modifier family of proteins, may modulate tumorigenesis by noncovalent interaction to MAD2. The inhibition of MAD2 function has been associated with chromosomal instability, a characteristic of many cancers. This paper deals with the protein-protein interactions between FAT10 and MAD2 using protein-protein docking studies. The overall study identifies the amino acid residues which are important for binding in FAT10. Further studies are aimed at designing new chemical entities which may be used as potent therapeutic leads.

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KEY WORDS

Chromosome instability; check point; FAT10; MAD2; docking studies; Protein-protein docking.

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[I] INTRODUCTION

Dysregulated cell-cycle control or apoptosis often leads to tumorigenesis. An instrumental role is played by ubiquitin and ubiquitin-related families of proteins in various cellular processes through modification of target proteins [1, 2].

Ubiquitin is a conserved 76 amino-acid polypeptide that plays role in conjugation of target proteins, thereby tagging "these proteins for degradation via the 26s proteosome pathway [3, 4]. This process of ubiquitylation, is an ATP dependent process that involves the sequential action of at least three different classes of enzymes. An E1 or ubiquitin activating enzyme, an E2 or ubiquitin conjugating enzyme, an E3 or ubiquitin protein ligase and in some cases an E4 chain elongation factor [5].

1.1. Ubiquitin-like proteins

A new group of proteins have been discovered recently that are related to ubiquitin or function similarly. These ubiquitin-like proteins can be divided into two groups, namely the ubiquitin - like modifiers (UBLs) and Ubiquitin-domain proteins (UDPs) [1].

Ubiquitin- like modifiers (UBL) modify other proteins by covalent formation of an isopeptide-bond with their target. These include Ubiquitin, SUMO-1/SMT3, SUMO-2, SUMO-3, NEDD8, ISG-15, FAT 10, HUB, Fau, An1a, An1b, APG12, URM1. Ubiquitin domain proteins (UDP) on the other hand are only structurally linked with ubiquitin and cannot become covalently conjugated to target proteins [1]. These include RAD23/HHR23A, B; DSK2, PLIC-1, PLIC-2/Chap1, XDRP1, BAG-1, BAT3/Chap2, Scythe, Parkin, UIP28/RBCK1, UBP6, Elongin B, Gdx.

FAT10 or diubiquitin belongs to UBL class of ubiquitin - like proteins. The FAT10 gene is identified as one of the genes in the MHC class I HLA-F locus of chromosome 6 [6]. It encodes an 18kDa protein containing 165 amino acid residues that has two ubiquitin- like domains, which are separated by a linker of 5 amino acid. The N-terminal domain is with 29% identity and C-terminal domain is with 36% identity with ubiquitin **[Figure–1]**.

Of the seven lysine-residues in ubiquitin four are conserved in both ubiquitin- like domains of FAT10. These lysine residues correspond with the Lys-27, Lys-33, Lys-48 and Lys-63 of ubiquitin. Among these lysine residues Lys-48 and Lys-63 of ubiquitin are important for polyubiquitin-chain formation. Also the diglycine motif of the very C-terminus of the C-terminal domain is conserved. Atypical of ubiquitin is the appearance of four cysteine residues in FAT10 [8].

Over-expression of the FAT10 gene was observed in the tumors of several cancers including gastrointestinal and gynaecological cancers [9]. Increased mitotic non-dysjunction and chromosome instability was observed in cells having high level expression of FAT10 protein [10]. Fat10 has been shown to bind

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noncovalently to the human spindle assembly checkpoint protein, MAD2 [11], a protein responsible for maintaining spindle integrity during mitosis [12].The inhibition of MAD2 function has been associated with chromosomal instability, a characteristic of many cancers [13,14].

Protein-protein interactions are integral to many cellular control mechanisms, because of the formation of protein–protein complexes in many biological events. Disruption of protein–protein interactions can cause biochemical diseases [15, 16]. In order to identify the way how proteins interact with each other, the structural details of these complexes at atomic level and their docking studies will be particularly important for elucidating functional mechanisms and designing inhibitors, and thus is a major goal of structural biology [17-19]. In the present study, we have carried out protein-protein docking studies between the two proteins i.e., FAT10 and MAD2.

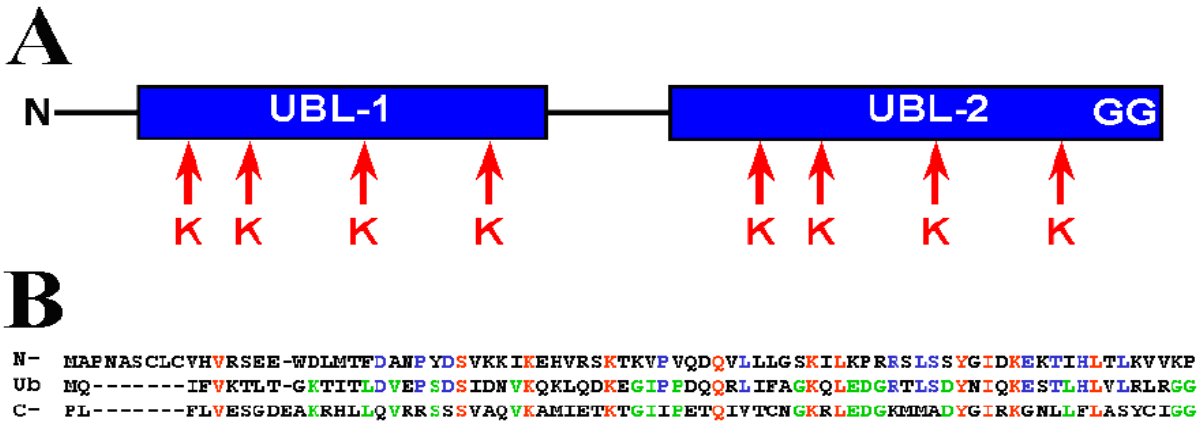

Fig: 1. Primary Structure of FAT10 and its sequence similarity to ubiquitin. (from Mark Steffen Hipp, 2005) [7]. (A) schematic diagram of FAT10 (B) sequence comparison of the N and C-terminal halves of FAT10 (N- / C-) with ubiquitin (Ub)

[II] MATERIALS AND METHODS

2.1. Sequence retrieval and template selection

The amino acid sequence of FAT10 is retrieved in FASTA format from SWISS PROT database followed by BLAST against PDB for template selection. The BLAST is used to find the similarity of the sequence to closest homologous proteins with known structures available in the PDB and identifies the structure with high identity and similarity to be employed as template for homology modeling.

2.2. Sequence alignment and model building

By extracting the sequence of template and target the alignment process is carried out by using ClustalW. Using "MODELLER 9.12" the 3D structure of FAT10 is generated. Energy minimization of the modeled structure is carried out by applying CHARMm force fields and steepest descent algorithm followed by conjugant gradient algorithm in DS until the convergence gradient is satisfied.

2.3. Model validation

2.3.1. Procheck

Procheck is used in validation of protein structure and models by verifying the parameters like Ramachandran plot quality, peptide bond planarity, bad non-bonded interactions, main chain hydrogen bond energy, C-alpha chirality and over-all G factor and the side chain parameters like standard deviations of chi1 gauche minus, trans and plus, pooled standard deviations of chi1 with respect to refined structures [20].

2.3.2. Prosa

This program compares *Z* scores between target and template structure which are a measure of compatibility between its sequence and structure. The *Z* score of the model should be comparable to the *Z* scores obtained from the template [21, 22].

2.3.3. Docking studies

Protein-protein docking studies of diubiquitin (FAT10) with its receptor, mitotic spindle check point protein (MAD2), were performed in GRAMM-X Protein-Protein Docking Web Server v.1.2.0 using the GRAMM global search algorithm [23]. The crystal structure of MAD2 with PDB entry 2V64 containing 205 amino acid residues at a resolution of 2.90 was taken from the PDB database.

[III] RESULTS

3.1. Sequence retrieval and template selection

The FASTA sequence of the FAT10 protein (1-165) taken from SWISSPROT database accession number: **O15205**, entry name: UBD_HUMAN, and protein name: **Ubiquitin D** was submitted to BLAST against PDB database. The BLAST results yield X-ray structure of 2ZVN from human having a sequence identity of 31% with a resolution of 3.00 A0. All the further procedures are carried out using MODELLER 9.12.

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3.2. Sequence alignment and model building

An essential input to a homology modeling program is the sequence alignment between the template-target pair. Alignment of FAT10 protein with the extracted sequence of 2ZVN was carried out using Clustal W. On the basis of this alignment as input, model of the FAT10 is built using "MODELLER 9.12." Ten molecular models of FAT10 are generated. The refinement process is carried out using DS by applying CHARMm force field and steepest descent method is applied with 0.001 minimizing RMS gradient and 2000 minimizing steps followed by conjugant gradient method till it reaches the satisfactory results for minimization. The energy refinement method gives the best conformation to the model **[Figure– 2]**.

3. 3. Model validation

The final refined modeled structure of FAT10 protein is analyzed by the Procheck and Prosa.

3.4. Procheck

Procheck is used to evaluate the overall stereochemical quality of the modeled protein by analyzing the overall residue by residue geometry and psi and phi torsion angles of Ramachandran plot showing residues with most favorable region (88.4%), generously allowed region (0.0%), additionally allowed region (10.2%), and disallowed region (1.4%) in comparison to 2ZVN template (80.2%, 0.8%, 19.2%, and 0.0%), respectively. The plot statistics are given in **[Table–1, Figure-3]**.

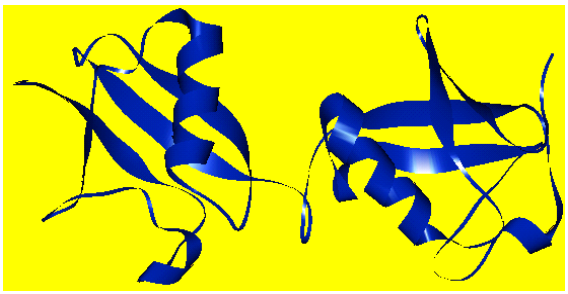

Fig: 2. Modeled FAT10 protein

Table: 1. The % of residues in the core region of the Ramachandran plot for the built FAT10 model and the template

Structure	Core	Allowed	Generous	Disallowed
2ZVN	80.2	19.2	0.8	1.4
FAT10	88.4	10.2	0.0	0.0

3.5. Prosa

Prosa was used for quality assessment of the model which revealed that the FAT10 model matched NMR region of the plot with *Z* score (-6.48) which is reliable to the *Z* score of the template 2ZVN (-7.37) . It signifies the quality of our model.

[IV] DISCUSSION

4.1. Docking studies

Protein-protein docking has been performed using GRAMM-X docking server which employs smoothed potentials, refinement stage, and knowledge based scoring [23]. It uses smoothed Lennard-Jones potential on a fine grid during the global search FFT stage, followed by the refinement optimization in continuous coordinates and rescoring with several knowledge-based potential terms. The extracted PDB structures 2V64 of MAD2 and FAT10 are taken for docking studies. The top 4000 grid-based predictions are subjected to a conjugate gradient minimization in continuous 6D rigid body space with the soft potential. The minimization accumulates many points, initially located on the grid, in a fewer local minima. One representative prediction for each minimum is stored and the number of initial predictions falling into this minimum is marked as the volume of the minimum. The average radius of such minima is 5 s. The local minimization of a smoothed landscape can be viewed as clustering on the original rugged Lennard-Jones landscape, and helps locate the protein binding funnel.

For each minimized prediction soft Lennard-Jones potential, evolutionary conservation of predicted interface, statistical residue–residue preference, volume of the minimum, empirical binding free energy and atomic contact energy are calculated. To eliminate predictions that are likely to be located far from the correct binding site, Support Vector Machine filter trained on a subset of the benchmark set is applied using the above mentioned set of potential terms. The remaining predictions are re-scored by a weighted sum of the potential terms. **Figure– 4** illustrates the top ranking pose of predicted MAD2-FAT10 complex formation that reveals the correct binding mode of the protein.

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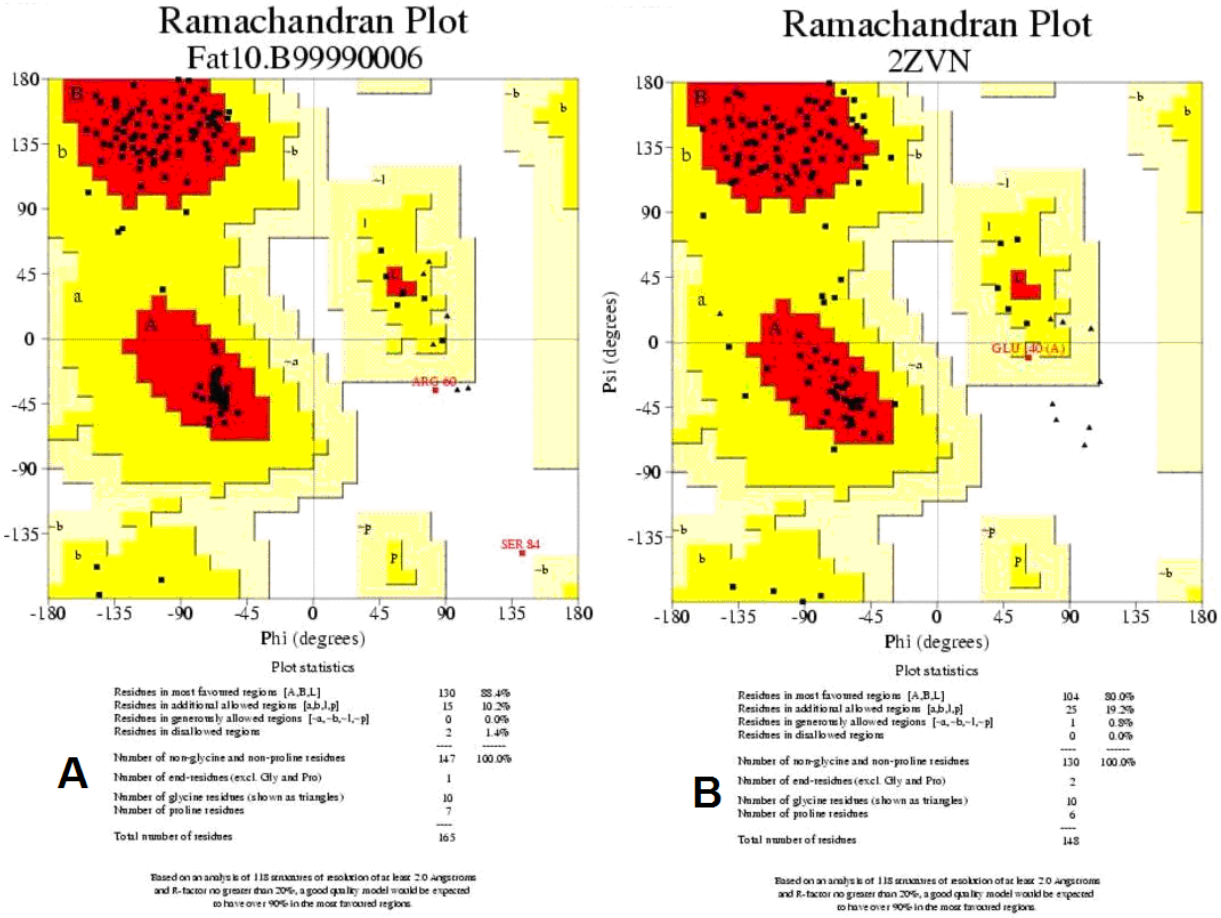

Figure: 3. A) Ramachandran's Map of FAT10 protein. B) Ramachandran's Map of 2ZVN protein

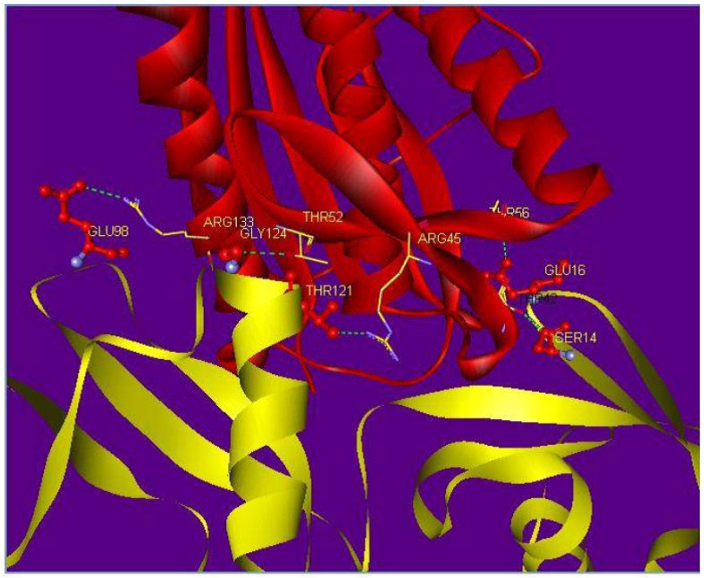

Fig: 4. The top ranking pose of predicted MAD2 (red colored flat ribbon shaped)-FAT10 (yellow colored flat ribbon shaped) complex formation illustrating intermolecular hydrogen bonding between the active site regions of the MAD2 and FAT10 complex. The amino acids on MAD2 involved in hydrogen bonding are represented in yellow colored stick shaped while the amino acids on FAT10 involved in hydrogen bonding are represented in red colored ball and stick shaped.

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Several intermolecular hydrogen bonds consistently form between the active site regions of the MAD2 and FAT10 complexes which are listed in **[Table–2]**. All these binding interactions lead to stability of complex thus preventing the function of mitotic check point function of MAD2. MAD2 (mitotic arrest-deficient 2) is a key mitotic spindle checkpoint protein which ensures that all of the chromosomes are properly attached to the mitotic spindle before the onset of anaphase [24]. It is activated by associating with unattached kinetochores. Activated MAD2 binds to Cdc20 and prevents the anaphase-promoting complex from ubiquitylating securin. As a result, anaphase is delayed until all of the kinetochores are attached by microtubules and the chromosomes are properly aligned along the metaphase plate [12, 25, 26]

Table: 2. Various binding interactions between the MAD2 and FAT10 complex

Sl. No	Intermolecular Hydrogen bonds	
	MAD2	FAT10
1.	THR 42	SER 14
2.	ARG 45	THR 121
3.	THR 52	GLY 124
4.	THR 56	GLU 16
5.	ARG 133	GLU 98
6.	SER 14	THR 42

[IV] CONCLUTION

The present study demonstrated the binding interactions of the MAD2-FAT10 complex through docking analysis. Docking studies reveals the correct binding mode and interacting amino acids. This protein – protein docking studies of MAD2 and FAT10 provides a great assistance in understanding structural details and may lead to the establishments of therapeutic approaches in designing of effective drugs for gastrointestinal and gynaecological cancers.

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CONFLICT OF INTEREST

Author declares no conflict of interest.

FINANCIAL DISCLOSURE

The work is not supported by any grant.

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RESEARCH ARTICLE OPEN ACCESS

BIODEGRADATION OF REACTIVE DYES BY AN ISOLATED BACTERIUM LYSINIBACILLUS SPHAE RICUS RSV-1

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ABSTRACT

A potential bacterial strain isolated from dyewaste effluent was characterized and identified as Lysinibacillus sphaericus RSV-1 based on biochemical and 16S ribosomal RNA gene sequence study. The strain was utilized for decolorization and degradation of various reactive dyes. Optimization of yeast extract concentration, pH, temperature, salinity and biomass was determined to be 0.5%, 7.0, 30˚C and 20% respectively. Effects of various nitrogen and carbon sources on decolorization of 100 ppm concentration of ten different mixed reactive dyes revealed, yeast extract, soya chunk powder, rice husk extract showed best nutritional supplement with 95±0.47, 89±0.94 and 92±1.63 percent decolorization within 2-48 hrs of incubation. Our isolated strain could be effectively utilized for the treatment real textile effluent containing high concentration of reactive dyes.

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KEY WORDS

Decolorization ; dyewaste effluent ; Lysinibacillus sphaericus; reactive dyes

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[I] INTRODUCTION

Textile effluent discharge is becoming a major environmental pollution in India particularly in Tirupur. During reactive dyeing processes, up to 50% of the dye that present in the original dye bath is lost to the wastewater that let in to nearby river without any treatment [1]. The reactive dyes are not degraded by the conventional wastewater treatment processes [2] are characterized by the presence of a nitrogen–nitrogen double bond (–N=N–), namely the azo group bound to aromatic groups [3]. Azo dyes are as recalcitrant in nature are not readily degraded by conventional methods [4]. More than 10–15% dye does not bind to fiber during color processing and release into the wastewater causing serious environmental pollution [5, 6]. There are several physical and chemical methods available but these have disadvantages of being very expensive, the formation of large amount of sludge and the emission of toxic substances [7]. In addition, the accumulation of concentrated sludge creates a disposal problem [8]. It is estimated that at least 100 tonnes of sludge will have to be disposed of every year. Many microorganisms has been using as bioremediation agents in the treatment of wastewater-containing textile dyes [9]. An advantage of using pure culture system ensures that the data are reproducible and that the interpretation of experimental observations is easier [10]. Many pure culture bacteria has been utilized for the treatment of reactive dyes such as, Pseudomonas sp. SUK1 for Reactive Red 2 [11], *Exiguobacterium* sp. RD3 for Reactive Blue 172 [12], *Rhizobium radiobacter* MTCC 8161 for Reactive Red 141 [13], *Pseudomonas aeruginosa* NBAR12 for Reactive Blue 172 [14] and bacterium KMK48 for the degradation of various sulfonated reactive azo dyes [15]. Bioremediation is a process in which the natural capacity of microbes is enhanced to degrade toxic chemicals and waste [16]. Several reports are available indicating that a variety of microbes have been involved in the bioremediation and biodegradation of dyes, which includes some bacteria such as, *Aeromonas hydrophila* [17], *Pseudomonas* sp SUK 1 [18, 19], *Exiguobacterium sp* RD 3 [12], *Pseudomonas* KF46 and *Kurthia* sp. [20], *Aeromonas hydrophila* [21] *Pseudomonas luteola* [22], bacterial consortium *(Aeromonas caviae, Proteus mirabilis* and *Rhodococcus globerulus)* [23]. Moreover, bacterial degradation is much faster than fungal degradation of textile dyestuffs [24]. Attempts have been made to decolorize the reactive mixed dyes using our previously isolated *Lysinibacillus sphaericus* strain [25].

[II] MATERIALS AND METHODS

2.1. Isolation of bacteria

Dyewaste effluent samples were collected from textile dyeing unit and CETP located in Tirupur. In our previous study, effective decolorizing strains were isolated based on primary, secondary screening and acclimatization study [25].

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2.2. Media preparation

Previously isolated strain was maintained on nutrient agar medium with the composition (g L-1) of pH;7.0 ± 0.02 Peptone(5.0); Yeast extract(2.0); Beef extract(3.0); NaCl (5.0); Agar (16.0). All the decolorization experiments were performed in Minimal Salt Medium (MSM) of pH 7.0 contained (g L-1) the following composition NaCl (1.0), $CaCl_2.2H_2O$ (0.1), $MgSO_4.7H_2O$ (0.5), KH_2PO_4 (1.0) and Na_2HPO_4 (1.0). In the present study all the experiments were performed with mixture of ten reactive dyes namely Yellow ME4GL, Blue RR, Red RR, Yellow RR, Red M5B, Blue MR, Deep Black RR, Yellow MERL, Red ME4BL and Golden Yellow MR. About 1000 ppm stock was prepared by adding equal amount of each dye.

2.3. Identification of the strain

A pure colony of the bacterium was grown in LB medium until log phase growth was obtained. The resulting growing bacteria were obtained with centrifugation. Genomic DNA from the isolate was extracted with the bacterial genomic DNA Isolation Kit (RKT09). Amplification was done using prokaryotes 16S rRNA specific forward Primer: 5'-AGAGTRTGATCMTYGCTWAC-3 and reverse Primer: 5'-CGYTAMCTTWTTACGRCT-3' (Sreekumar and Krishnan 2010). The reaction mixture contained 1µl of template DNA, 400 ng of each forward and reverse primers, 4 µl of dNTPs (2.5 mM), 10 µl of 10x DNA polymerase assay buffer, 1.5mM of MgCl2 as final concentration, 1 µl of Taq DNA polymerase enzyme (3U/ µl), water to make the final volume as 100 µl. The PCR conditions were initial denaturation of 94˚C for 5 min, denaturation of 94˚C for 30 sec, annealing of 55˚C for 30 sec, extension of 72˚C for 2 min and final extension of 72˚C for 15 min. Number of cycles were 35. The reaction was carried out in Thermal Cylcer ABI2720 (Chromous biotech). The sequencing mixture contained 1 µl of Template (100ng/ µl), 2 µl of Primer (10pmol/ µl), 3 µl of Milli Q water and the conditions were initial denaturation of 96˚C for 1 min followed by denaturation of 96˚C for 10 sec followed by hybridization 50˚C for 5 sec finally elongation of 60˚C for 4 min for 25 cycles. The nucleotide sequence was determined automatically using Big Dye Terminator v3.1 Cycle sequencing Kit in the ABI PRISM 3130- Genetic Analyzer with universal primers. DNA sequence analyses were performed using the BLAST search and was aligned with sequences from other bacterial sequences retrieved from GenBank database of NCBI. An alignment of sequences was done by CLUSTAL W. The phylogenetic tree was constructed using Mega 4.0 software.

2.4. Optimization of physico chemical parameters

Effect of yeast extract concentration (0.05, 0.1, 0.2, 0.4, 0.8 % (w/v), pH (3, 5, 7, 9 and 11), Temperature (28 ºC, 30 ºC, 37 ºC, 45 ºC, and 50ºC), Salinity (1, 3, 5, 7, 9 %) and biomass concentration (4, 8, 12, 16, and 20 %) on decolorization was studied. The final concentration of mixed dye was 100ppm. The culture was inoculated previously in nutrient broth medium incubated at 30ºC for 12 hours. After incubation, cells were pelleted (6000×g for 20 min) and the growth was monitored spectrophotometrically at 600nm. About 1.0 OD at 600 nm was utilized for optimization parameter study. The medium to inoculums was maintained at 50:1 ratio. All the experiments were performed in MSM and triplicates were maintained for all the experiments.

2.5. Effect of supplemental carbon and nitrogen sources as co-substrates

To study the effect of carbon and nitrogen sources on decolorization, MSM was prepared by adding different carbon and nitrogen sources such as (mannitol (1%), inositol (1%), Corboxy methyl cellulose (CMC) (1%), lactose (1%), sucrose (1%), starch (1%), dextrose (1%) as a carbon source and yeast extract (0.5%), ammonium nitrate (1%), ammonium sulphate (1%), ammonium chloride (1%) and urea (1%) as nitrogen source. In addition, to make the process economically feasible, appropriate concentration of rice husk extract (10%), wheat husk extract (10%), straw waste (1%), sugarcane juice (1%) and Soya chunk power (1%) were mixed with 100 ml distilled water individually and autoclaved at 121˚C for 20 min, MSM was made up with appropriate concentration of each substrate.

2.6. Effect of yeast extract and soya chunk powder on decolorization of individual as well as mixed reactive dye by RSV-1

To study the effect of yeast extract and soya powder (selected based on previous study result) on decolorization, MSM was prepared by adding 1000 ppm concentration of each Yellow ME4GL, Blue RR, Red RR, Yellow RR, Red M5B, Golden Yellow MR, Deep Black RR, Yellow MERL, Red ME4BL, and Blue MR and maintained in appropriate optimised conditions. Spectrophotometric reading was done for the analysis of percent decolorization of individual dyes where as for the mixed dyes, colour of the samples was determined using HACH DR 2800 spectrophotometer and the results were expressed as (pt/co).The percentage of decolorization was calculated as follows:

% Decolorization = Initial absorbance - Observed absorbance/Initial absorbance x 100

[III] RESULTS

3.1. Isolation and Identification of bacteria

Dye waste effluent samples were collected from textile dyeing unit and CETP located in and around Tirupur, Tamilnadu, India. About 112 isolates were obtained from Primary screening and 50 isolates from secondary screening. The effective strain having decolorization ability of four mixed reactive dyes up to 2700 ppm concentration with 52% decolorization within 49 hrs of incubation was selected from acclimatization study was utilized in the present study(data not shown)25. Biochemical and 16S ribosomal RNA gene sequence was performed for identification of the strain. The strain was identified on the basis of cell shape, cell arrangement, nutritional characteristics, physiological and biochemical characteristics. The morphological, physiological, and biochemical characteristics of the isolated strain RSV-1 was listed in [Table– 1]. Gram Staining result showed that the strain RSV-1 was Gram positive and showed negative results for indole, methyl Red, V-P Test, citrate utilization test, TSI Test, glucose, lactose, was sucrose, xylose, mannitol, arabinose, hydrolysis of starch, catalase and nitrate reduction test, where as positive for oxidase and gelatin liquefaction test. Presence of growth was observed in temperature (10–50°C) and NaCl (2.0–7.0%). The strain was motile when observed under inverted microscope. The cell morphology was spherical rods, smooth coherent cluster, convex and Opaque. The culture was light grey in colour. 16S rRNA sequence was analyzed using BLASTn and multiple sequence alignment was performed using CLUSTAL W. Phylogenetic tree was constructed using MEGA 4.0 [Figure–1]. Result revealed that the strain RSV-1 identified as *Lysinibacillus sphaericus* which was found to be 99% identical to *L. sphaericus* (JF343178) among selected taxonomic group

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of other bacillus species. The strain was deposited in the GenBank database under accession number JF502569. A bootstrap value of 500 indicates that a branching pattern is confirmed in all the resampling, whereas a bootstrap value of 60 indicates that the branching pattern is reproduced only in 60% of the resamplings.

Table: 1. Morphology, Cultural and Biochemical Characterization of *Lysinibacillus sphaericus* RSV-2 strain

Biochemical and culture conditions	*Lysinibacillus sphaericus* RSV-2
Gram staining	Gram-positive
Indole	-
Methyl red	-
V-P test	-
Citrate Utilization Test	-
TSI Test	-
Glucose	-
Lactose	-
Sucrose	-
Xylose	-
Mannitol	-
Arabinose	-
Hydrolysis of starch	-
Catalase	-
Oxidase	+
Gelatin liquefaction	+
Nitrate reduction	-
Phenylalanine deaminase	+
Growth at temperature (10–50°C)	+
Growth on NaCl (2.0–7.0%)	+
Cell type (shape)	Spherical rods
Color	light grey
Surface	Smooth
Arrangement	Coherent cluster
Density	Opaque
Elevation	Convex
Motility	Positive

3.2. Optimization of Yeast extracts concentration

Effects of yeast extract concentration on maximum decolorization was studied by adding the medium with 0.05, 0.1, 0.2, 0.4, 0.8 % of yeast extract along with 100 ppm of mixed dye in 100 ml containing MSM. The maximum decolorization was obtained in medium containing 0.8% of yeast extract **[Figure– 2A]**. The percentage decolorization of RSV-1 was found to be in the range of 50.77-98.75 in increasing concentration of yeast extract respectively. The highest biomass was found to be 1.409 OD at 0.8% yeast extract concentration. Considering the high cost, further experiments were performed by using 0.4% yeast extract.

3.3. Optimization of pH

The effect of pH on decolorization was investigated in the pH ranges of 3, 5, 7, 9, and 11. It was found that change in pH significantly affect the decolorization rate. The isolates showed good growth at pH 7 and 9, while at pH 3 and 5 were found to be inhibitory for the growth. Bacterial cultures generally exhibit maximum decolorization at pH values near 7.0 **[Figure– 2B]**. The percentage decolorization of RSV-1 was found to be 94.37 and 91.99 at pH 7 and 9 respectively at 36th hour of incubation. The biomass OD 1.128 and 0.681 obtained for pH 7 and 9 respectively.

3.4. Optimization of temperature

Temperature plays an important role in microbial growth and enzyme activity; it is one of the most important parameter taken into consideration for the development of biodecolorization processes. The influence of temperature on decolorization was performed in the temperature range of 26ºC, 30ºC, 37ºC, 45ºC, and 50ºC. The maximum percentage of decolorization was found to be 99.61, 98.64 and 91.01 in the temperature range of 30ºC, 37 ºC, and 45ºC respectively by RSV-1. The biomass OD was found to be 1.00 -1.5 in that temperature [**Figure–2C**]. The temperature conditions studied in the present investigation were selected based on the average high and low temperatures of the concerned zone. Optimal temperature to decolorize mixed azo dye for RSV-1 strain was found to be 30°C.

3.5. Optimization of NaCl concentration

Effect of NaCl concentration on decolorization was studied by adding the medium with the range of 1, 3, 5, 7, 9 % concentration of NaCl. The optimized pH, and Temperature of 7.0 and 30ºC was kept as constant. When the concentration of NaCl increase there was decrease in decolorization activity. Our isolate RSV-1 achieved maximum decolorization and the biomass OD of 82.72, 82.95 and 0.826, 0.760 at 1 % and 3 % NaCl concentrations, respectively [**Figure–2D**].

3.6. Optimization of biomass concentration

Effect of biomass concentration on decolorization was performed by adding the medium with 1.0 OD at 600nm culture of 4, 8, 12, 16, and 20 % (v/v) concentration inoculum. When the concentration of biomass increased, the growth and percentage decolorization also increased. At 36th hours, the percentage removal of dye by the isolate RSV-1 was found to be 50.04, 67.44, 91.20, 94.57 and 99.07% at inoculum concentrations 4, 8, 12, 16 and 20% respectively and the OD was 0.9 and 1.2 at 16 and 20%, respectively [**Figure- 2E**)].

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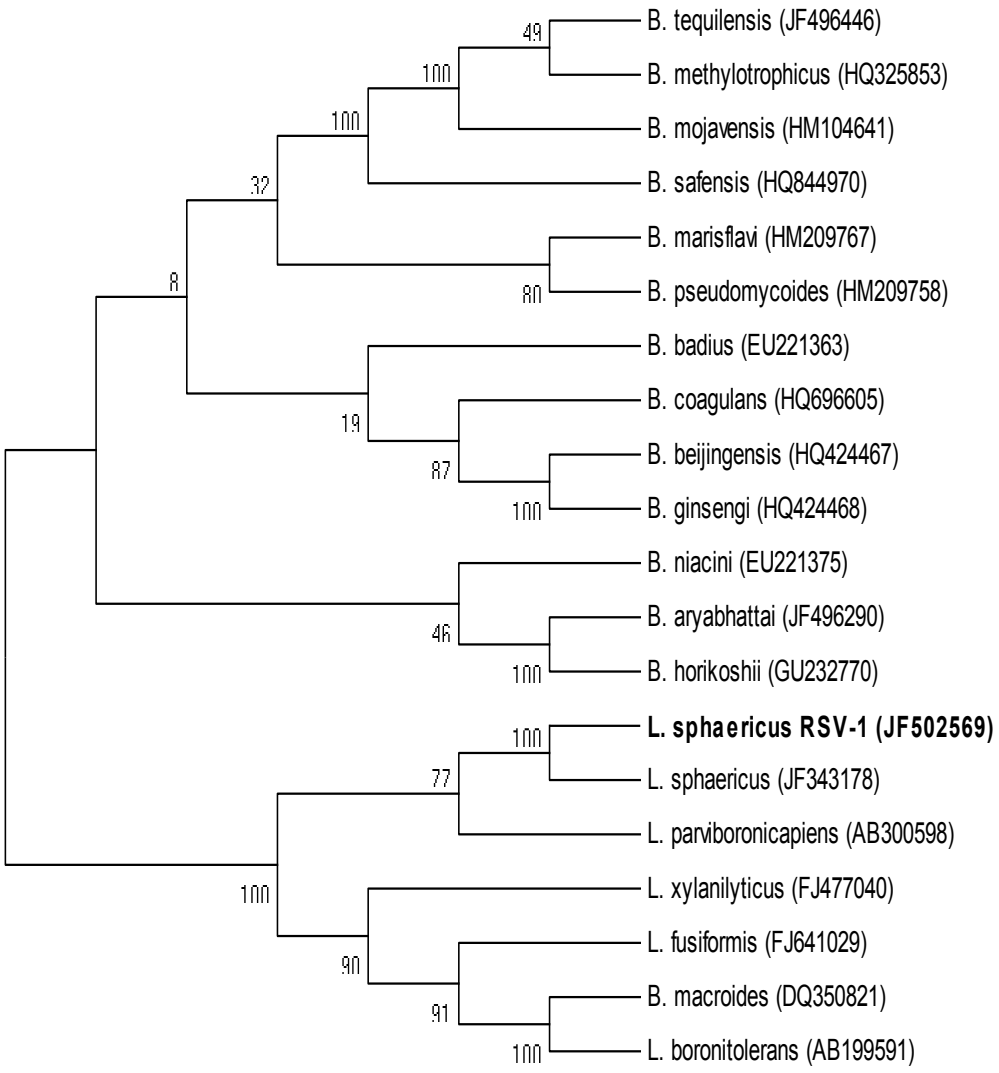

Fig: 1. Phylogenetic analysis of 16S rRNA sequence of *Lysinibacillus sphaericus* strain RSV-1 using MEGA-4.0 software. The percent numbers at the nodes indicate the levels of bootstrap support based on neighbor-joining analyses of 500 replicates. Brackets represent sequence accession

3.7. Effect of supplemental carbon and nitrogen sources as co-substrates

In order to check the growth and decolorization efficiency of the strain, the MSM was supplemented with various carbon and nitrogen sources. The decolorization and growth was absent in MSM, since it contains no added C or N sources. About 3-5% decolorization was observed when dextrose and sucrose was used and 12% was observed when starch was used as carbon source. There was no decolorization in lactose and CMC supplemented medium. The strain couldn't able to grow and decolorize the dye incorporated in inorganic nitrogen supplements such as ammonium nitrate, urea, ammonium chloride and ammonium sulphate. The strain effectively utilized yeast extract and decolorized the dyes up to 95% within 2 hr. Other than yeast extract, one more substrate served better nutrient supplement with 89% decolorization with in 3 hrs of incubation was soya chunk powder. Agricultural waste also served better decolorization rate, when rice husk extract was utilized and the percentage was found to be 92% in 48 hrs **[Table– 2]**. The effective decolorization may due to the strain be able to utilize the husk particles as a supportive material and also utilized the nutrients present in it for growth. The wheat husk served only 36% of decolorization while straw waste and sugar cane juice served only 15 and 12% decolorization.

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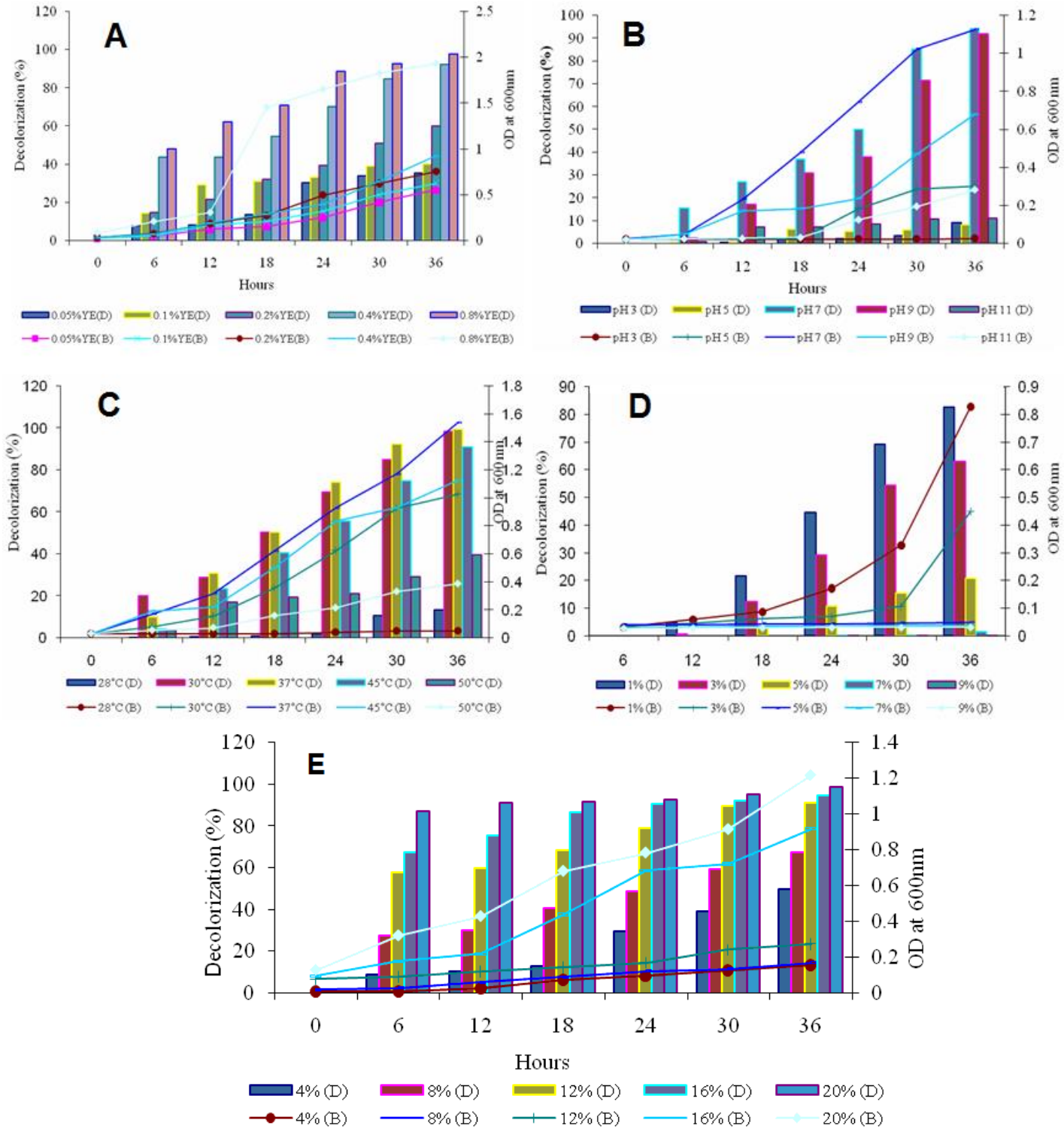

Fig: 2. A) Effect of yeast extract concentration on decolorization by RSV-1, B) Effect of various pH ranges on decolorization by RSV-1. C) Effect of various temparatures on decolorization by RSV-1. D) Effect of NaCl concentrations on decolorization by RSV-1. E) Effect of biomass on decolorization by RSV-1

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Table: 2. Effect of supplemental carbon and nitrogen sources on decolorization of textile dyes by RSV-1 strain

Substrate	RSV-1	Time (h)
MSM	ND	48
MSM + Dextrose (1%)	03±0.81	48
MSM + Lactose (1%)	ND	48
MSM + Sucrose (1%)	05±0.47	48
MSM + Mannitol (1%)	ND	48
MSM + Inositol (1%)	ND	48
MSM + CMC (1%)	ND	48
MSM + Starch (1%)	12±0.81	48
MSM + Ammonium nitrate (1%)	ND	48
MSM + Ammonium sulphate (1%)	ND	48
MSM + Ammonium chloride (1%)	ND	48
MSM + Urea (1%)	ND	48
MSM + Yeast extract (0.5%)	95±0.47	2
MSM + Soya chunk power (1%)	89±0.94	3
MSM + Wheat husk[a] (10%)	36±0.94	30
MSM + Rice husk[a] (10%)	92±1.63	48
MSM + Straw waste[b] (1%)	15±0.47	48
MSM + Sugar cane juice (1%)	12±2.49	48

ND=no decolorization also absence of growth; CMC=Carboxy methyl cellulose; MSM=Minimal salt medium. [a]The extracts were prepared by mixing 20g of each rice and wheat husk with 100ml of boiled distilled water(stock) and 10% of each substrate was made in MSM taken from the stock.these were autoclaved at 121˚ C for 20 min as mentioned by (Jadhav et al., 2008) with little modification

3.8. Effect of yeast extract and soya chunk powder on decolorization of dyes by RSV-1

The strain was inoculated in MSM prepared with optimized concentration of yeast extract, pH, temperature, dye concentration and inoculum concentration of 0.4%, 7.0, 30˚C, 1000 ppm and 20% of 1.0 OD (v/v) respectively. Static condition was maintained throughout the experiment. Strain RSV-1 could effectively decolorized the follwing dyes namely Deep Black RR, Yellow MERL, Red ME4BL, and Golden Yellow MR in the range of 90-99% decolorization. The decolorization percentage was within the range of 80-90% when Red M5B and Blue RR were used. Red RR achieved 76% of decolorization. Moderate decolorization was obtained in the range 40-65% when Yellow RR and Blue MR were utilized by the strain. The strain could achieve only 21% of decolorization when Yellow ME4GL used, this may be due to complex structure of the dye. No significant difference was observed when soya chunk powder was used as a co-substrate [**Table– 3**]. Structure of some of the study dyes were given in [**Table– 4**].

Table: 3. Effect of yeast extract and soya chunk powder on decolorization of dyes by RSV-1

Name of reactive dyes	λ_{max} (nm)	% Decolorization	
		Yeast extract medium	Soya chunk medium
Yellow ME4GL	418.8	21±0.94	10±0.47
Blue RR	576.9	83±2.62	81±0.94
Red RR	503.2	76±1.69	74±0.47
Yellow RR	442.8	65±0.00	79±0.47
Red M5B	508.5	88±1.69	82±1.69
Blue MR	564.5	45±1.63	52±1.69
Deep Black RR	566.3	92±1.14	86±0.00
Yellow MERL	404.5	98±1.14	87±1.69
Red ME4BL	418.8	98±0.47	94±1.69
Golden Yellow MR	414.6	98±1.14	87±0.47
Mixed dye	514.9	67±0.47	63±1.69

Values are mean of three experiments ± SEM. There is no significant difference were observed when yeast extract and soya chunk powder were used as a substrate since p>0.05 by one way ANOVA with Post Hoc test

[IV]DISCUSSION

Tirupur is an Indian textile town which constitutes many dyeing and bleaching units situated in the upstream. Tirupur serves as one of the major exporters of textiles. The industrial pollution have affected not only the surface water but also the soils and ground water even though industrial units have either constructed or connected to effluent treatment plants, the level of treatment has not been satisfactory at most of the places. Even today, some of the unit operate illegally and let the untreated effluent in to nearby river. It has caused serious impact on agriculture, livestock and fisheries. The effluents of CETP contain the mixture of various dyes, complex substances (from dyeing process), since it is necessary to collect the microbes from their native environment. The dyeing and bleaching units use a variety of toxic chemicals, including hydroxides, hydrochloric acid, sulphuric acid and sodium nitrate. The effluents caused various health problems such as skin allergies and lung infections. The Tirupur municipal hospital reports widespread incidence of skin diseases and pulmonological disorders. Biological methods are simple to use and low cost is involved in operation [26]. The biological treatment has many advantages over physical or chemical methods. Hence the survey was undertaken for the isolation of effective microorganism for the effective treatment of textile dye effluent. Effluent discharge area of textile industry is observed to be rich in biodegradation microbial flora. These can thrive there because of their metabolic adaptability.

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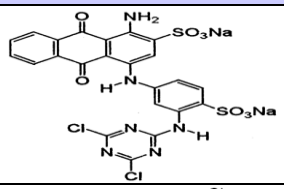
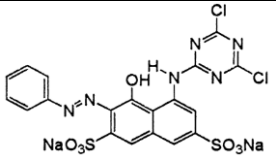
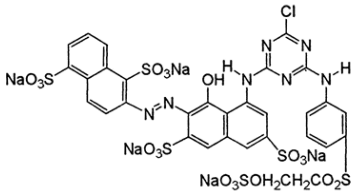
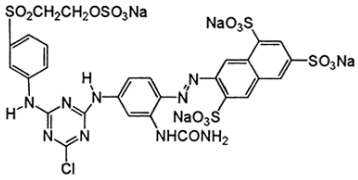
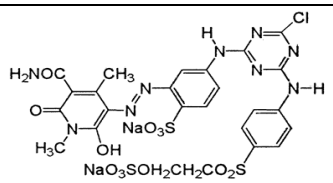
Biodegradation of textile dyes by living forms occurs naturally in the natural habitats [27]. Considering the volume generated and the effluent composition, textile industry wastewater is rated as the most polluting among all industrial sectors [28]. Hundreds of dyeing industries are in Tirupur, an Indian town in Tamil Nadu. About 610 dyeing units located on the bank of the river Noyyal release untreated effluents into the river. The high bacterial counts reflect that the textile dyeing effluents are good sources of nutrients to facilitate the growth of certain bacteria [29]. Azo dyes are selected from the list of dyes mostly used by dye industries at Tirupur and Karur districts in Tamilnadu, India. These dyes are regularly used in textile industries and untreated wastewater is always let out into adjacent channels. The isolation of efficient dye decolourisation bacteria from the samples collected from dye contaminated soil and wastewater indicates the natural adaptation of these microorganisms to survive in the presence of the toxic dyes [30]. In Mathew and Madamwar studies, among the various organic and inorganic nitrogen sources used, yeast extract was the best nitrogen source for efficient decolorization of RFB by the SV5 bacterial consortium. This may be owing to the metabolism of yeast extract, which is considered essential for the regeneration of NADH [31]. The strain *Bacillus endophyticus* VITABR13 with strong decolorizing ability was isolated from textile effluent to decolorize the textile Azo dye Acid Red 128 (100mg/l) [32]. Dawkar et al. studied Decolorization of RHE7B was 100% under static conditions and 0% under shaking conditions. But the growth of Bacillus sp. VUS was more in shaking conditions (44 mg/l) as compared to static conditions (33 mg/l) [12].

Table: 4. Name of the decolorized dyes with structure

Name of Dye	Structure	Class of dye	Molecular weight
Reactive Blue MR		anthraquinones	681.39
Reactive Red M5B		Single azo class	615.34
Red ME4BL		Single azo class	1136.32
Reactive Yellow MERL		Single azo class	1026.25
Yellow ME4GL		Single azo class	818.13

Originally, *Lysinibacillus sphaericus* genus was *Bacillus* sp. and was transferred into this genus as *Lysinibacillus* sp. in 2007 [33]. *Lysinibacillus* are ubiquitous and numerous in soils possess the ability to catabolism various compounds and xenobiotics. *L. sphaericus* has been reported to degrade p-nitrophenol, caprolactam and dichloromethane [34-36]. Strain

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Lysinibacillus sp ZB-1, the first one of this genus, was found to possess the ability to metabolize fomesafen. *Lysinibacillus* are able to survive under extremely harsh conditions, which make them ideal candidates for bioremediation of contaminated environments [37]. Wan et al. isolated a new bacterial strain from activated sludge has been identified as *Lysinibacillus sphaericus* based on its morphology, physiochemical properties, and the results of 16S ribosomal RNA (rRNA) gene sequence analysis. This new bacterial strain uses ethanethiol as both carbon source and energy source [38]. *Lysinibacillus* sp. could degrade fomesafen (one of the diphenyl ethers, is specifically used for early post-emergent control of broad leaf weeds in soybean and bean) was isolated from contaminated agricultural soil, and identified as based on the comparative analysis of 16S rRNA gene. The optimum temperature for fomesafen degradation by strain was 30 ˚C [37]. *B. sphaericus* is having mosquito larvicidal activity and it is active against Culex and Anopheles mosquito larvae. An advantage of *B. sphaericus* over other larvicidal bacterial strains like *Bacillus thuringiensis* var *israelensis* can grow even in polluted water [39]. Russell et al. studied the carbohydrate metabolism in mosquito pathogenic strain *B. sphaericus* 2362. This bacterium was found to be unable to transport glucose or sucrose into the cell and it lacked glucokinase and hexokinase activities [40]. *Lysinibacillus sphaericus* produced light grayish colour colonies and effective growth was observed in pH 7.

The metabolism of yeast extract is considered essential to the regeneration of NADH that acts as the electron donor for the reduction of azo bonds. It had also been found that increasing yeast extract concentrations (from 0 to 10 g/l) resulted in higher decolorization rates, and the decolorization rates reached a plateau as yeast extract was higher than 8 g/l [17]. When compare to beef extract and nutrient broth medium, yeast extract medium was more appropriate for the decolorization of dye Navy blue 2GL by Bacillus sp. VUS [42]. Bacterial consortium consists of Bacillus cereus (BN-7), *Pseudomonas putida* (BN-4), *Pseudomonas fluorescence* (BN-5) and *Stenotrophomonas acidaminiphila* (BN-3) capable of completely decolorizing C.I. Acid Red 88 (AR-88) (20 mg/l) in 24 h, whereas individual cultures took more than 60 h to achieve complete decolorization of the added dye. It was able to decolorize 78% of C.I. Acid Red 88, 99% of C.I. Acid Red 119, 94% of C.I. Acid Red 97, 99% of C.I. Acid Blue 113 and 82% of C.I. Reactive Red 120 dyes at an initial concentration of 60 mg/l of mineral salts medium (MSM) in 24 h [43]. Effect of different concentrations of yeast extract in the range of 0-0.15% (w/v) in MSM broth on the decolorization efficiency of HM-4 was evaluated. In the absence of any yeast extract supplement in MSM, only 34% of colour removal was observed whereas complete decolorization of the dye was achieved at 0.1% (w/v) of yeast extract in medium in 12 h [17].

It is thought that the pH effect may be more likely related to the transport of dye molecules across the cell membrane, which was considered as the rate limiting step for the decolorization [44]. Repeated addition of dye aliquots in decolorized medium by bacterial consortium RVM 11.1 was studied, which is significant for its commercial application also the rate of decolourization of individual dyes depends on structural difference of the dyes. [45]. It has been indicated that neutral pH would be more favorable for decolorization of the azo dye. In the case of live bacterial cells, the optimum pH for color removal is often at a neutral pH value or a slightly alkaline pH value. About 91% decolourization of 300 ppm Reactive Red BS dye within 5.5 h was decolourized by *Pseudomonas aeruginosa* [46]. Reactive Red 180 was decolorized by *Citrobacter sp*. CK3 with the concentration of (200 mg/l), resulting in approximately 95% decolorization within 36 h, and could tolerate up to 1000 mg/l of dye [47]. The pH tolerance is quite important because reactive azo dyes bind to cotton fibers by addition or substitution mechanisms under alkaline conditions and high temperatures [48]. The optimal pH and temperature for the decolorization of RED RBN (1000ppm concentration) by the strain Proteus mirabilis were 6.5-7.5 and 30-35˚C. The strain could decolorize the dye within 20 h of incubation. [49]. Most textile and other dye effluents are produced at relatively high temperatures and hence temperature is an important factor [50]. At 37°C the bacterial consortium showed maximum decolorization of Ranocid Fast Blue Dye RFB, followed by at 30°C. This may be owing to a greater production of enzymes and optimal growth conditions of the consortium SV5 for its dye-decolorizing ability. The decolorization at this optimal temperature may be owing to higher respiration and substrate metabolism. This also demonstrates that decolorization of the dye was through the microbial reaction, which relies on optimal temperature, and not by adsorption, where the temperature effect was not great [6]. The optimal temperature and pH for the decolorization of azo-dye reactive red 22 by *Pseudomonas luteola* was found to be 37˚C and 7-9 respectively. Yeast extract and tryptone were strongly enhanced the decolorization while glucose concentration retarded decolorization of reactive red 22 [51]. A novel bacterial species identified as *Exiguobacterium* sp. RD3 degraded the diazo dye reactive yellow 84A (50 mg/l) within 48 h at static condition, at 30˚C and pH 7 [27]. Wastewaters from textile processing and dye stuff manufacture industries contain substantial amounts of salts in addition to azo dye residues [52]. Salt concentrations up to 15–20% have been measured in wastewaters from dyestuff industries [53]. An inoculum size, beyond 10% was not very significant in decolorization was observed [31]. Decolorization activity of *Bacillus* sp. has high (86.72%) in 4% of inoculums [54].

The decolorization rate increased with increase in the inoculum size, reaching maximum (2.53 mg l-1) at 20% (v/v) inoculum size. However, beyond 20% (v/v) inoculum size, rate of decolorization did not vary significantly [55]. Triphenylmethane group of dyes was decolorized intracellularly by a strain *Kurthia* sp under aerobic conditions with 98% color removal [56]. The reduction of azo dyes depends on the presence and availability of cosubstrates, because it acts, as an electron donor for the azo dye reduction [57]. Strain *Pseudomonas aeruginosa*

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strain BCH capable of decolorizing Direct Orange 39 (50 mg/l) within 45 ± 5 min, with 93.06% decolorization ability was isolated from sediments contaminated with dyestuff [58]. It is thought that in case of complex substrates such as extract of rice husk and rice straw, the strains could convert and degrade them, producing some volatile organic acids or alcohols (such as acetic acid and ethanol), which acts as electron donors and apparently induces the reductive cleavage of azo bonds [59,60]. Decolorization was favoured by strictly anaerobic conditions and highly proteinaceous media. [61]. Sugarcane extract is eco-friendly as compared to others reduction method likes electrochemical and ultrasonic energy [62]. Azo dye decolorization by mixed, as well as pure, cultures generally required complex organic sources, such as yeast extract, peptone, or a combination of complex organic source and carbohydrate [17, 43]. The supplementation of cheap co-substrates (e.g., extracts of agricultural wastes) could enhance the decolorization performance of consortium GR consisting of *Proteus vulgaris* NCIM-2027 and *Micrococcus glutamicus* NCIM-2168 could rapidly decolorize and degrade commonly-used sulfonated reactive dye Green HE4BD and many other reactive dyes [63]. In contrast, the bacterial reduction of the azo bond is usually nonspecific and bacterial decolourization is normally faster. Microbial decolourization and degradation has appeared as an environmentally friendly and cost-competitive alternative to chemical decomposition processes although this bacterium has shown greater dye degradation ability as compared to other bacteria. The chemical structures of the dyes greatly influence their decolourization rates, and the decolourization efficiency is limited to several azo dye structures [64].

Metabolism of the yeast extract is considered essential for regeneration of NADH, which is the electron donor for azo bond reduction [65]. Azo dyes generally contain one, or more sulphonic-acid groups on the aromatic rings, which might act as detergents, thereby inhibiting the growth of the microorganisms. Such dyes may affect DNA synthesis since it has also been reported that dyes are inhibitors of the nucleic acid syntheses, or cell growth [66, 17]. The effect of carbon (1 g%) and nitrogen (0.5 g%) sources on the decolorization ability of the bacterial isolates M1 (Bacillus cereus) and M6 was studied by Modi et al. [67] Decolorization of azo dye Red 3BN by two bacterial species Bacillus cereus and *B. megaterium* was studied by Praveen Kumar et al. Optimal condition for B. cereus was found to be 1% sucrose 0.25% peptone, pH 7, 37°C and 8% inoculum and that for *B. megaterium* was found to be glucose 1%, 0.25% yeast extract, pH 6, 37°C and 10% inoculum. Extent of decolorization recorded by B. cereus under ideal conditions was 93.64% and that by *B. megaterium* was 96.88% [69]. *Pseudomonas pseudomallei* 13NA and *Citrobacter* sp. Decolorized both triphenylmethane and azo dyes by a single species of bacterium [70]. *Bacillus* sp. VUS decolorized azo dye Navy blue 2GL in 48 h at static anoxic condition in yeast extract medium. The time required for 94% decolorization of 50 mg/l dye was 48 h. Yeast extract was best medium for faster decolorization than other media [42, 68]. B. cereus performed well because they are nutritionally versatile and carries an efficient enzymatic system for the cleavage of azo bonds, which cause rapid decolourization of different azo dyes and thus they are able to biodegrade many natural and synthetic organic compounds. This could be a consequence of natural adaptation of the organism as the sample from which the bacterial isolate was obtained was highly contaminated with dyes [71].

A strain *M. glutamicus* was applied to decolorize a mixture of ten reactive dyes (Reactive Green 19A, Reactive Yellow 17, Reactive Red 2, Reactive Orange 4, Reactive Blue 171, Reactive Orange 94, Reactive Blue 172, Reactive Red 141, Reactive Red 120, and Reactive Blue 59) at a concentration of 50 mg/l and 37˚C under static condition. Decolorization was found to be 63% within 72 h [72]. There are also several studies describing decolorization of reactive dyes mediated by pure bacterial culture such as, *Pseudomonas sp*. SUK1 for Reactive Red 2 [11], *Exiguobacterium* sp. RD3 for Navy Blue HE2R (Reactive Blue 172) [12], *Rhizobium radiobacter* MTCC 8161 for Reactive Red141 [13], *Pseudomonas aeruginosa* NBAR12 for Reactive Blue 172 [14] and isolated bacterium KMK48 for the degradation of various sulfonated reactive azo dyes [15]. Decolorization of Reactive Red 2, Reactive Red 141, Reactive Orange 4, Reactive Orange 7 and Reactive Violet 5 was observed by the unidentified bacterium, KMK 48. Complete decolorization (100%) of Reactive Red 2, at the concentration of 200 and 1000 mg/l was observed after 30 h. However, 200 mg/l of Reactive Red 141 was decolorized within 24 h, whereas decolorization of 1000 mg/l was achieved after 30 h. Moreover, 200 mg/l of Reactive Orange 4 was decolorized completely after 30 h whereas; with 1000 mg/l decolorization was observed at 36 h. The time taken for total decolorization of Reactive Orange 7, having the concentration of 200 mg/l was 18 h whereas that of 1000 mg/l was found to be 24 h [15].

The difference in decolorization rate between Acid Red 27 and Reactive Red 2 is possibly caused by the presence of the triazine group, as the report of Van der Zee showed that dyes containing triazine group were among the dyes that reduced at slowest rates [73]. The growth characteristics and capacity of *E. faecalis* to decolorize Acid Red 27 was better and higher than that on Reactive Red 2 because this dye has a triazine group in its chemical structure. The more complicated structure of Reactive Red 2 was a factor that could decrease growth quality and capacity of *E. faecalis* to decolorize azo dye [74]. The bacterial consortium NBNJ6 showed rapid decolorization of all dyes within 24–35 h at 37 ˚C. Culture could decolorize upto 250 mg/l of Reactive Black B, Reactive Blue 172, Reactive Violet 5R, Reactive Red 5B and Reactive Black RL, within 30–35 h at 37 ˚C in the range of 85–90%. Food colors like Ponceau 4R (500 mg/l) Raspberry Red (1000 mg/l) Tartrazine (300 mg/l) and Sunset Yellow FCF (300 mg/l) were decolorized within 24 h in the range of 84–95%. The rate of decolorization of individual dyes varied [75].

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[IV] CONCLUTION

The use of a pure culture system ensures reproducible data, and thus interpretation of experimental observations becomes easier. The present study reveals that the isolated strain could effectively utilized for the treatment of real textile effluent containing high concentration of reactive dyes. The optimum pH and temperature for maximum decolorization ability was found to be 7-9 and 30°C. Moreover, 0.4 % of yeast extract or soya chunk powder was served best nutritional supplements

FINANCIAL DISCLOSURE

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CONFLICT OF INTEREST

Author declares no conflict of interest.

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