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TREATMENT OF PULP AND PAPER MILL EFFLUENT BY BACTERIA - *Pseudomonas putida*

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Abstract

At present, a large amount of water required for paper production and various chemicals has been identified in effluents, which is produced at different steps of paper making in paper mills. The pulp and paper industry is typically related to pollution difficulties related to high biological oxygen demand (BOD), chemical oxygen demand (COD), color, suspended solids, lignin and chlorinated compounds. Several studies have been made on eliminate these difficulties of pulp and paper effluents, the problem still continues. On the basis of pollutant, identified three *Pseudomonas* putida, strains (MTCC 2252, 9782, and 1194) procured from Microbial Type Culture Collectoion (MTCC), Chandigarh, India and were used in experiments. The strains were efficient for utilization of phenol & cresol, co metabolism of chlorinated compound and decolonization. After 24hrs of incubation, the removal efficiency of these three strains and their combinations for color, biological oxygen demand and chemical oxygen demand varied from 40% to 45%, 35% to 42% and 30% to 40% respectively. The pH values ranged from 6.81 to 8.65. Storage places are limited so fast removal of contaminants from waste water is really helpful for pulp and paper mill industry. Therefore, the development of this finding into a large scale offers an attractive technology for waste water treatment.

Keywords

Chemical oxygen demand, Biochemical oxygen demand, Color

1. INTRODUCTION

An enormous industrial growth has taken place throughout the world in the past few decades. It has become so vast that, the environment has totally changed from what it was earlier. Due to increasing human needs, the level of pollution in environment has raised to devastating extends leading to disastrous consequences. Pollution today is found in each and every thing that we need the most viz. air, water, soil, etc. Water being one of the most important natural resources, is required in huge amount to fulfill all human needs. Apart from personal usage, the amount of water utilized by various industries is very large. Pulp and paper mills are one of the main water and energy intensive industries as it is sixth largest water polluting sector [1]. Typically in India around 75% of total fresh water supplied to pulp and paper industries emerges as waste water. In comparison to other industries fresh water requirement in pulp and paper industry is quiet high (150-200 m3) per ton of product [2].

The The problems associated with pulp and paper mill effluents are pH, color, and high levels of Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solids (SS), and Absorbable Organic Halides (AOX) etc.

Paper manufacturing process release chlorinated lignosulphonic acids, chlorinated resin acids, chlorinated phenols (trichlorophenol, trichloroguicol, tetrachloroguicol, dichlorophenol, dichloroguicol and pentachlorophenol), and chlorinated hydrocarbon in the effluent, are the major contaminants formed in the effluent of pulp and paper mill [3-6]. Physicochemical characteristics of pulp and paper mill effluent was mentioned in literature (Raj etal., 2014).

Pulp and paper industries generate 220-380 m3 of highly colored and potentially toxic wastewater for every ton of paper produced (Badar and Farooqui, 2011). The main component of this type of wastewater is chlorolignin. For ages, lignin has been well known for its resistance to microbial degradation because of its high molecular weight and presence of various biologically stable carbon-to-carbon and ether linkages [7]. But of late, few bacteria have been reported to be able to degrade lignin [8]. However, not much work has been undertaken towards lignin degradation by bacteria. In addition, the chlorophenolic compounds formed in chlorine bleaching are toxic, persist, bioaccumulation, and transform into other compounds which are more hazardous. Government agencies mark the standards for the discharge of wastewater into the environment; the BOD standard of 30 mg/L for discharge on inland surface water and 100 mg/L for disposal has been notified under the environment protection rule.

Various studies have reported detrimental effects of pulp and paper mill effluent on animals living in water bodies receiving the effluent. Microbial biodegradation is carried out by different organisms like Bacteria, Fungus, and Algae [9-10]. 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 [11]. 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 [12]. Bioremediation process involves detoxification and mineralization, where the waste is converted into inorganic compounds such as carbon dioxide, water and methane [13]. When compounds are persistent in the environment, their biodegradation often proceeds through multiple steps utilizing different enzyme systems or different microbial populations [14-15].

Of late, biological treatment came in to scenario in these systems, where wide varieties of microorganisms including fungi, actinomycetes, and bacteria have been implicated in biodegradation of lignin via an oxidative process [16-18]. Fungi contain lignin peroxides, manganese peroxides, and so forth, which can effectively degrade lignin, but they are unable to work efficiently under extreme environmental conditions, namely, high temperature, pH, and presence of toxic chemicals which usually exist in the treatment plants. In addition, fungal filaments cause structural hindrance, so its utilization is not feasible for biological treatment of pulp and paper industry effluent [18].

In the above context, that effluent needs appropriate treatment prior to release in the environment; other-wise, it represents a major environmental problem. The main problem of industry is storage places and did not have any fast treatment way of waste water. On the basis of screening and Identification, study on three *Pseudomonas putida*, strains which were efficient for utilization of phenol & cresol, co metabolism of chlorinated compound and decolonization, were examined in laboratory scale to evaluate them for the treatment of Paper industry effluent. In particular their potential in decolonization, the reduction of the COD and the increase in the inorganic chloride content were analyzed.

2. Materials & Method

2.1. Chemicals, Reagents, Glassware, and Media. All chemicals, reagents, and media used in the present study were of analytical grade and obtained from Hi-Media, India and Sigma Aldrich, India. The laboratory glass wares used were washed with detergents and rinsed with distilled water then autoclave at 121ºC and 15 psi pressure for 15 minutes, prior to use.

2.2. Sample Collection. Wastewater samples were collected from effluent treatment plant of hard wood based paper mill. Samples were collected and stored at 4◦C till further use.

2.3. Screening & Collection. Different bacterial isolates were screened for their ability to degrade pulp and paper wastewater. From the literature, three *Pseudomonas putida* (MTCC 9782, MTCC 2252, and MTCC 1194) strains were screened and procured from Microbial Type Culture Collection (MTCC), Chandigarh.

2.4. Formulation of Consortia. The nature of pulp and paper industry effluent is quite complex as it contains a number of organic components, for example, lignin, tannic acid, resin, cellulose, and hemicelluloses which are difficult to be degraded by a single bacterial isolate. So, there is a need for the formulation of effective microbial consortium which cans biodegrade the effluent in minimum time period. So the bacterial strains (MTCC 9782, MTCC 2252, and MTCC 1194) were formulated of consortia on the basis of their rapid growth on nutrient media (Growth medium-3).

2.4 Screening of consortia. The inoculum (mother culture) was prepared by inoculating one loopful of all individual bacterial isolates in 25 mL of sterilized nutrient broth. The inoculated broths were incubated in an orbital shaker at 25°C and 37°C for 16–24 h respectively their growth temperature so as to obtain actively growing mother cultures. The above-mentioned actively growing cultures were inoculated separately in 100 mL of sterilized nutrient broth and incubated at 25°C and 37°C, 150 rpm for 16-20 hrs. On the basis of these three microbial strains growth kinetic study, achieving the maximum growth (Optical density 1.6) at 16-20 hrs. All the isolates were taken in 50 mL graduated centrifuge tube and centrifuged at 10000 rpm for 20 minutes at 4°C. After centrifugation, supernatant was discarded and the pellet was washed twice with 50 mM sodium phosphate buffer. Bacterial pellet was inoculated in 100 mL of wastewater sample.

2.5 Optimization of Parameters. Flasks were incubated for 24 hrs at 25°C, 37°C and 30°C and 150 rpm for strains and consortia. After 24 hrs, the sample was taken and estimated as per standard methods (APHA). Different parameters like pH, temperature, aeration, color, lignin, COD, BOD and so forth were optimized to achieve maximum reduction. Effluent generate from paper mills is highly toxic so it is difficult to treat it as such by bacteria. It is mixed in different Bacteria: Effluent ratio (1:10, 1:20 and 1:40).

The pulp and paper effluent is deficient in nitrogen (N) as well as phosphorus (P) constituents, so nitrogen and phosphorus were supplemented during wastewater treatment. Various parameters (temperature, pH, and aeration) were standardized in order to get efficient treatment in less duration.

After the parameters were optimized, the experiments were repeated in order to check the reproducibility among the results with the procured bacteria and designed consortium of bacteria.

3 Results

3.1. Characterization of Wastewater Sample

In the present study, samples were collected from hard wood based Paper Mill. Different parameter load of wastewater varies from time to time due to use of various raw materials used in pulping and paper making process so that the average value of COD, BOD, Color, Lignin, Phenolics and pH of the primary clarifier was 757mg/L, 441mg/L, 716.66 PCU, 90g/L, 8.36g/L and 8.7 respectively.

3.2. Optimization of Parameters

The optimization of different parameters, namely pH, color, lignin, phenolics, COD and BOD were studied. While studying temperature was maintained as per given but in case of consortia, the average temperature was maintained. The pH value ranging from 6.5 to 8.2, maximum reduction was observed at 7.0 to 7.5. Treatment time, Aeration Agitation was constant at all the time.

3.3. Ratio of effluent and inoculum

In order to seen the reduction in pollution parameters, different dilutions were carried out. The dilution was repeated with varying ratio of bacterial strains and wastewater ranging from 1:40, 1:20 and 1:10. Maximum reduction in color by *Pseudomonas putida* (Figure 1, 2, and 3) was observed when of bacterial strains and wastewater was used in 1:10.

Table 1: Values of various treated and untreated effluent parameters after 24 hrs

Properties	**Primary Effluent**	**MTCC 1194 Strain**			**MTCC 2252 Strain**			**MTCC 9782 Strain**		
		40:1	**20:1**	**10:1**	**40:1**	**20:1**	**10:1**	**40:1**	**20:1**	**10:1**
pH	7.35	7.58	7.85	7.86	7.4	7.4	7.3	7.2	7.1	7.5
Color (PCU)	560	544	532	506	488	438	397	438	395	356
Lignin (g/L)	257	184	180	167	179	176	174	167	154	138
Phenolics(g/L)	8.36	6.14	6.01	5.81	6.75	6.35	5.98	6.54	6.21	5.93
COD (mg/L)	1180	680	636.8	612.8	845	765	705	756	723	691
BOD (mg/L)	590	246	190	163	502	478	413	485	412	379

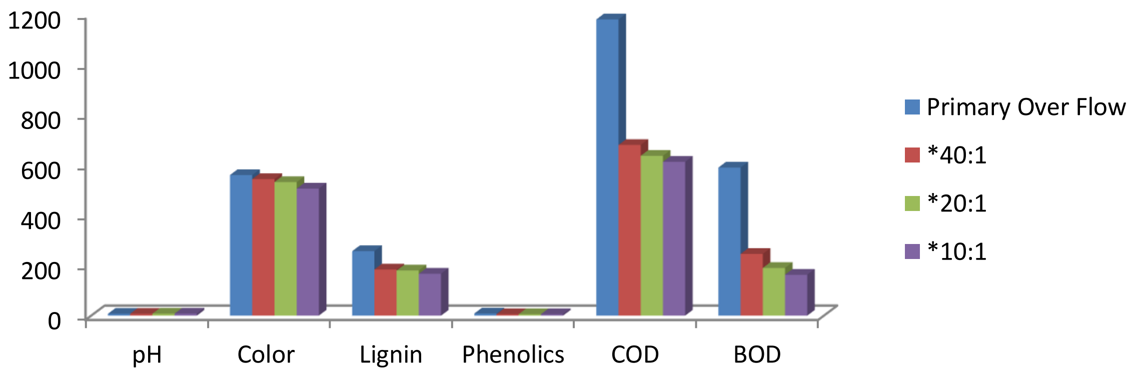

Figure 1: Comparative study of different parameter pH, color, lignin, phenolics, COD and BOD using different ratio of bacterial strain (MTCC 1194) after 24 hrs as per primary overflow.

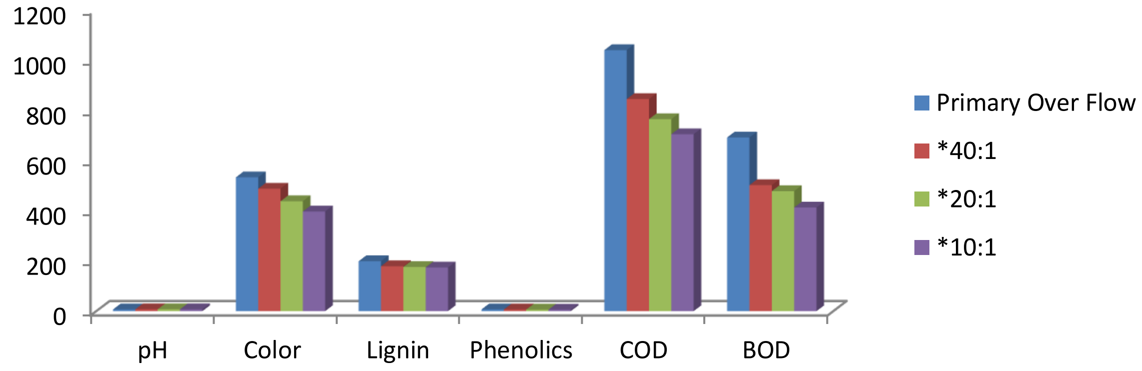

Figure 2: Comparative study of different parameter pH, color, lignin, phenolics, COD and BOD using different ratio of bacterial strain (MTCC 2252) after 24 hrs as per primary overflow.

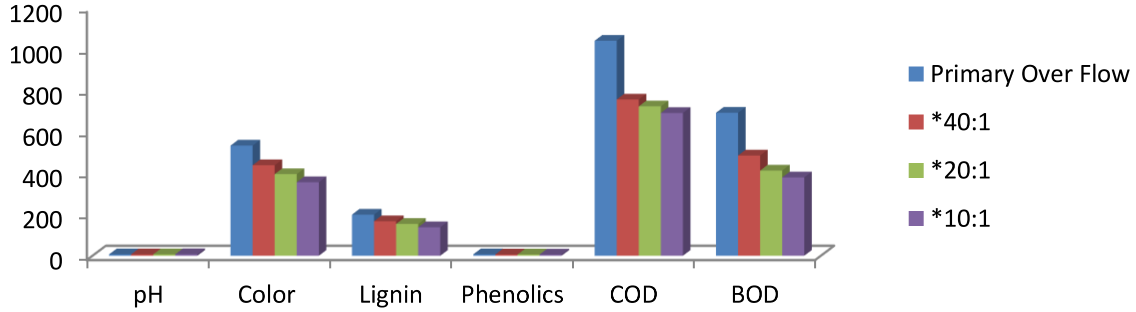

Figure 3: Comparative study of different parameter pH, color, lignin, phenolics, COD and BOD using different ratio of bacterial strain (MTCC 9782) after 24 hrs as per primary overflow.

3.4. Nitrogen, Phosphorus and Carbon Supplements

To check weather color and COD reduction could be enhanced by addition of Carbon, Nitrogen and Phosphorus nutrients, experiment was performed with and without carbon, nitrogen and phosphorus. The experiment was performed by adding carbon, nitrogen and phosphorus in effluent and inoculum ratio (1:40, 1:20 and 1:10), and result related that color and COD reduction could be achieved (Figure 4 and 5).

3.5. pH

Effect of pH on color and COD reduction was also observed by varying pH of wastewater from 6.8 to 8.0, and the results showed that best color and COD reduction could be achieved in the flask with the pH 7.0 (Table 3).

3.6. Color and COD

While studying the effect of color value ranging of bacterial strains individually different. The ranging from 544 PCU to 307 PCU, maximum reduction was observed at 10:1, effluent: bacterial culture ratio. Result reveled that COD reduction could be achieved 35-38 % (Table 1, 2 and 3). However, the COD reduction was observed 40-42 % in presence of carbon, Nitrogen and Phosphorus (Figure 4 and 5).

Table 2: Value of various parameters using bacterial consortia after 24 hrs.

Properties	Primary Effluent	Consortia of MTCC 1194, 2252, 9782 strains		
		40:1	20:1	10:1
pH	8.7	7.6	7.2	7
Color (PCU)	717	548	468	402
Lignin (g/L)	269	178	153	133
Phenolics(g/L)	7.58	7.01	6.89	6.63
COD (mg/L)	757	440	499	480
BOD (mg/L)	441	336	132	398

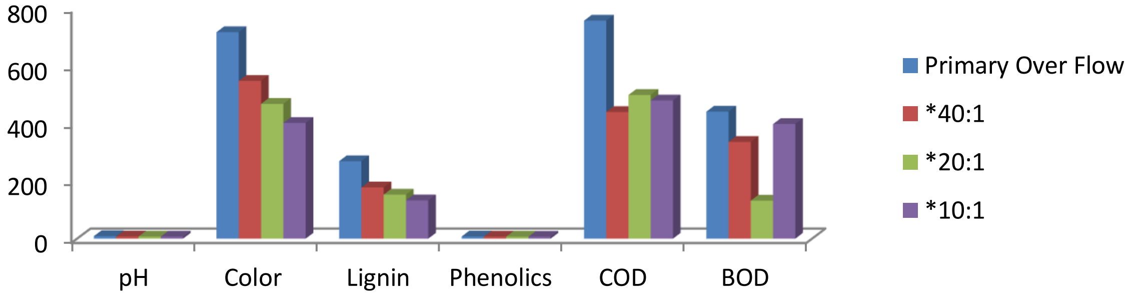

Figure 4: Comparative study of different parameter pH, color, lignin, phenolics, COD and BOD using different ratio of bacterial consortia after 24 hrs as per primary overflow.

3.7. Repeatability and Reproducibility of Results after optimization of Parameters

Figure 4 showed the maximum reduction in different parameters like color and lignin up to 38-45% at different effluent: bacterial ratio and figure 5 also showed the reproducibility of the parameter ranges at the optimized conditions (Treatment time, Aeration, Agitation and Supplement (Glucose, Phosphorus and Nitrogen) doses was 24 hrs, continuous, 150rpm respectively.

Table 3: Percentage reduction of various parameters using bacterial consortia after 24 hrs.

Properties	Primary Effluent	Consortia of MTCC 1194, 2252, 9782 strains		
		40:1	**20:1**	**10:1**
pH	8.1	7.4	7.1	7.2
Color (PCU)	443	311	298	236
Lignin (g/L)	191	109	101	92
Phenolics(g/L)	6.92	6.31	6.01	5.23
COD (mg/L)	728	415	387	356
BOD (mg/L)	313	274	238	216

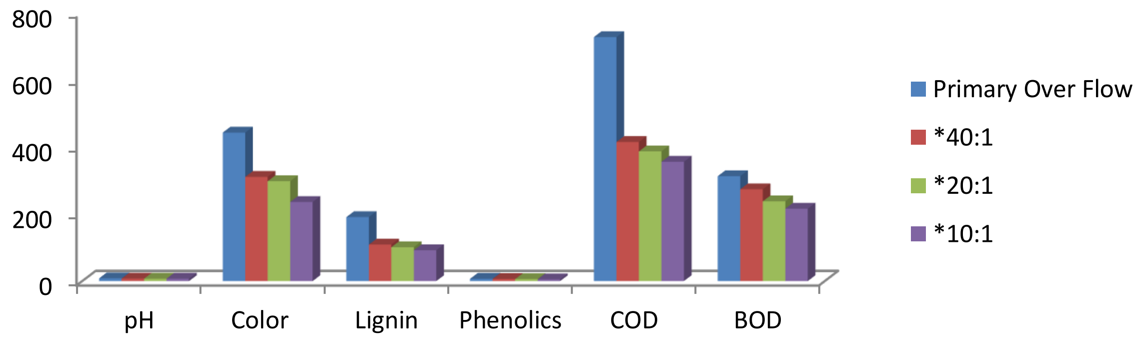

Figure 5: Comparative study of different parameter pH, color, lignin, phenolics, COD and BOD using different ratio of bacterial consortia after 24 hrs as per primary overflow.

4. Discussion

Pulp and paper mills are responsible for water pollution due to lack of chemical recovery system which might be due to insufficient infrastructure and high initial investment. So that storage of paper mill effluent always a big problem. Lignin and cooking chemicals produce from paper making process, which have deleterious effect on land and natural water bodies. Treatment of such a polluting stream is a big question among the researchers. Some researchers used the physiochemical treatment processes such as electrochemical, ozonation, coagulation adsorption flocculation and membrane filtration technology to treat the effluent. These technologies are efficient, but all of them are expensive for pulp and paper mills to implement for the treatment of effluent. So that Biological treatment of wastewater is also explored using various microorganism like bacterial species, fungus species etc.

In case of fungus, Freitas et al. [19] used white rot and soft rot fungal species for treatment of the effluent from Kraft pulp mills and showed COD reduction of 74–81% after 10 days. Wingate et al. [20] explored purified fungal cellobiose dehydrogenate for color remediation of pulp mill effluent and could remove up to 50% color after 4 days. But, fungal treatment of pulp and paper mills effluent was not feasible because fungi are unable to proliferate under extreme environmental conditions (high pH, temperature, and oxygen limitation) that are present in effluent treatment plant of pulp and paper mill effluent and fungi has at least 5 to 10 days for effluent treatment that is not feasible for pulp and paper mill industry due to storage capacity.

Table 4: % reduction change by the Consortia of MTCC 1194, 2252, 9782 strains Treated Effluent (At 24 hrs.) with respect to Primary Effluent.

Properties	Consortia of MTCC 1194, 2252, 9782 strains		
	40:1	20:1	10:1
pH	8.6	8.3	7.8
Color (PCU)	29.8	32.7	46.3
Lignin (g/L)	42.9	47.1	51.0
Phenolics(g/L)	8.8	13.2	24.8
COD (mg/L)	42.9	46.8	51.1
BOD (mg/L)	12.4	23.9	30.9

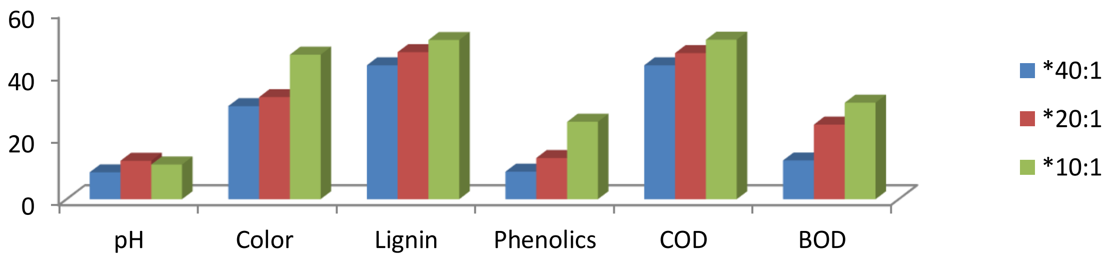

Figure 6: Reduction % change of pH, color, COD and BOD with respect to primary over flow after 24 hrs.

In order to have efficient treatment system, it is imperative to reduce COD, BOD, color, and so forth in minimum retention time. Therefore, bacteria are more efficient to degrade organic substance in minimum time period.

In the present study, different bacterial strains of *Pseudomonas putida* (MTCC 2252, 9782, and 1194) were indentified from literature and procured from Microbial Type Culture Collection (MTCC), Chandigarh, India. The experimental results showed that the removal of pollutants was influenced by all culture conditions of different strains and consortia. Through the number of experiments, it was found that every single strains of *Pseudomonas putida* have not paper mill's desired efficiency in 24 hrs treatment time. But in the case consortia of *Pseudomonas putida* strains give 40-45% reduction in color and lignin and 30-35% reduction in COD and BOD in 24 hrs treatment time. Experiment were repeated with consortia of *Pseudomonas putida* strains were used in different ratio of culture and untreated effluent to check whether their synergism might aid better color reduction. Based on the results, table 2 showed the maximum reduction efficiency in all parameters at 10:1 (untreated effluent: culture) ratio with *Pseudomonas* putida. Consortia of *Pseudomonas* putida was further optimized and found to have good potential to biodegrade the effluent generated from pulp and paper mills.

5. Conclusion

The result of this findings and literature suggest a great potential for *Pseudomonas sp.* In laboratory scale experiments with *Pseudomonas* putida strains have the ability to use color lignin and reduced the value maximum by 40-45% of untreated effluent by 24Hrs incubation. They were able to remove 35-40% of COD and BOD under optimized condition of pH, temperature and inoculums density. Through this study, we find maximum efficiency of *Pseudomonas* putida strains and their consortia have 40-45% color and lignin reduction in 24 hrs treatment time.

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