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ORIGINAL RESEARCH ARTICLE

Reutilization of industrial waste water for the production of Penicillin by Fermentation broth

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ABSTRACT

The main objective of the present study was to found the alternative sources for the use of water in fermentation. A lot of waste water was released after fermentation. This waste water could again be used as water sources for fermentation. In this work, attention would be focused on the use of varying amount waste water for penicillin production. The penicillin production was significantly high at low pH is given below detailed.

Key words: Penicillin, Fermentation, *Penicillium chrysogenum* and pH.

1. INTRODUCTION

The biosynthesis of penicillin is catabolically repressed by metabolic carbohydrates and carboxylic acids [1]. The synthesis is easier in the presence of phenyl acetic acid as the whole carbon and energy source [2]. The penicillin is a filamentous microorganisms consisting of a multi compartment structure of morphologically heterogenous hyphae. The pharmaceutical significance of this product is still growing especially due to the third and fourth generation 6-APA and 7-ADCA derivatives [3]. For this reason, a concentrated continuing quest for cost improvement in penicillin fermentation still focuses on better cultures, less expensive raw materials and their improve utilization [4]. The benefit of this effects and more clear understanding of the physiological condition for the penicillin biosynthesis path way. So far, many works have been done to find alternative cheap sources for the expensive raw materials in penicillin fermentation. Attention has been focused on alternative carbon sources [5]. Works have also been done on alternative nitrogen sources for penicillin fermentation [6]. There are no references in the literature to penicillin production by using waste water sources for fermentation. A lot of waste water in released after fermentation. This waste water can again be used as a water sources for fermentation. The objective of the present study was to investigate the use of varying amounts of waste water for penicillin production.

2. MATERIALS AND METHODS

Culture used

Penicillium chrysogenum ATCC 16520 cultures was obtained from American type culture collection.

For seed medium

Sucrose, Cotton seed meal, Calcium carbonate, Potassium dihydrogen sulphate and Diammonium sulphate.

For production medium

Cotton seed meal, Lactose, Ammonium sulphate, Dipotassium sulphate, Phenoxy acetic acid, Calcium carbonate and Potassium dihydrogen phosphate.

Revival of culture

The ampoule containing the spores of *Penicillium chrysogenum*, ATCC 16520 was cut opened aseptically. The contents were dissolved in 10 ml of sterile dissolved water to make a spore suspension and stored at 4 ± 1^0C (The suspension can also be distributed in 2 ml volume into small test tube, stopper with cotton plugs and refrigerator as tube cultures). One tube culture was the removed and 0.5 ml of spore suspension was further serially diluted upto10^6 dilution. From each diluted suspension, 0.1 ml was pipetted out and placed on LCS agar plates in triplicates under aseptic condition by spread plate method. All the

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plates were incubated at 25^0C for 12 days until the colonies reach 4 - 7 mm in diameter with heavy sporulation.

Substrate

The substrate was used as such and it was also used by mixing it with reverse osmosis water. Thus, form each substrate four type of samples were prepared as follows.

1) 100% of sample
2) 75% of sample with 25% of reverse osmosis water.
3) 50% of sample with 50% of reverse osmosis water.
4) 25% of sample with 75% of reverse osmosis water.

Preparation

The culture was inoculated aseptically in 75 ml of seed medium contained in 500 ml Erlenmeyer flasks and incubated at 25^0C in a shaker at 200 rpm for 66 hours. After incubation, the pH, PMV were checked and was observed in the microscope.

Fermentation medium preparation

C.S.M	27.5 g/l
Lactose	120.0g/l
AMS	12.0g/l
$K_2 SO_4$	1.5g/l
PoAA	10.0g/l
$CaCO_3$	10.0g/l
$KH_2 PO_4$	0.5g/l
pH	6.6

Three ml of the seed medium culture was added to 35 ml of the formation medium in the 500 ml Erlenmeyer flask. It was incubated at 25^0 C in shaker at 220^0 rpm for 120 hours. After incubation, the pH, PMV were checked. Then, the culture was filtered and the filtrate was subjected to HPLC to know the activity of *Penicillium chrysogenum*. Microscopic observation also done.

3. RESULTS

In the present investigation, it has been observed that the SPIC industrial effluent was treated using various chemicals such as Alum, chetizon and ferric chloride and also the effluents was already certain treatment made by the industry. Using the treated waste water the penicillin was produced. The pH value and the nitrogen content were estimated. The raw water samples contain the TDS are 0.25, 0.08, 0.27 and 0.74 mg/l for partially, fully treated and alter and before pH corrected water respecting. The Alum treated water observed the TDS was 0.03, 0.06, 0.14 and 0.17 mg/l for partially fully treated and after and before pH corrected both respectively. In the chitozon treated water TDS contain 0.19, 0.10, 0.19 and 0.23 mg/l for the different water samples (Table - 1). The observed values of TDS in ferric chloride treatment were 0.11, 0.07, 0.14 and 0.14 mg/l for partially, fully treated, after and before ph corrected broth respectively. Among the three chemical treatment, alum treatment was used for further studies. The alum treated different water samples contain pH value was 7.5 and 7.45 for partially fully treated water and 7.46 at 1.83 for after and before pH corrected broth respectively.

The alum treated various water samples contain the nitrogen levels 648.29 and 534.84, for partially and fully industrial treated water and 2389.30 and 203.37 for after and before pH corrected broth respectively. The COD values of alum treated different water samples were 2,400 and 3,000 for industrial partial and fully treated water samples and 25,000 and 60,000 for after and before pH corrected water respectively. The sulphuric content present in the alum treated different water samples were 13.83 and 17.69 for partial alum fully industrial treated water samples and 96.69 and 150.88 for after and before pH corrected broth respectively. The total dissolved solids content of alum treated water samples were 0.06 and 0.25 mg/l for full and partially treated water samples and 0.35 and 0.74 mg/l for after and before pH corrected broth. The growth of penicillin content in the partially treated water was 4949, 5320, 7406 and 8589 for 100%, 75%, 50% and 25%, respectively. Whereas in fully treated water samples, penicillin growth was 9658, 12328, 14623 and 17293 for 100%, 75%, 50% and 25%, respectively (Table - 1). The penicillin content was recorded in after pH corrected broth was 1298, 2298, 5917 and 8450 for 100%, 75%, 50% and 25% respectively. In the before pH corrected broth contain the penicillin level was 5.3, 7.2, 12.2 and 15.3 for 100%, 75%, 50% and 25% respectively.

4. DISCUSSION

Penicillin was one of the first antibiotics to be discovered. 70 years later, one of the most important in term of both therapeutic use and annual volume of production. In the present investigation, comparative penicillin yields was high at fully treated industrial waste waters at low pH from 6.32 to 6.69 for various diluted concentration (100 %, 75%, 50% and 25%). The total nitrogen content was 648.29 ppm. It has been noted that the nitrogen present in recycled water can stimulate the activity of microorganisms noted that the organic and inorganic nutrients in treated effluents that had a high carbon to nitrogen ratio

stimulate the activity of microorganisms. The reduced pH (Menthol level) of the treated waste water has recorded the significant level of penicillin production [7]. The present investigation has showed that the possibility of using this mycelium as an excellent nitrogenous raw material and the high quantity of mycelium needed for a fermentation. It could be interesting to determine how many times the mycelium could be recycled in tank fermentation without sacrificing yield. It has showed that fermentation broth and treated water could be recycled in an efficient way in the fermentation process.

The presence of rich mycelium content in the waste water was found to be a high growth activity. Similar results reported by Gosh [6]. APHA [8] have suggested that phenyl acetic acid is industrially used as the side chain precursor for the production of penicillin. Szarka [9] have also explained that the penicillin can oxidize some phenyl alkanes to phenyl acetic acid with high efficiency for production of penicillin.

5. CONCLUSION

So, we were concluded that the use of industrial waste water to produce the penicillin significantly high at low level pH and the mycelium, *Penicillium chrysogenum* was very essential for production of penicillin with the high sources of nitrogen.

Table 1: Variations of penicillin, mycelium content and pH value in the different treated water samples

S. No	Samples	Concentration (%)	Penicillin content	Mycelium Content	pH
	Control		37450	35	5.9
1	Partially treated water	100	4949	15	7.2
		75	5320	15.5	7.4
		50	7406	16	7.2
		25	8589	17.2	6.91
2	Fully treated water	100	9658	18.3	6.69
		75	12328	20.1	6.50
		50	14623	21.4	6.42
		25	17293	25.3	6.32
3	Spent Broth after pH correction	100	1290	7.1	7.8
		75	3398	12.3	7.4
		50	5917	16.4	7.23
		25	8450	16.6	7.25
4	Spent Broth before pH correction	100	-	5.3	8.03
		75	1328	7.2	7.72
		50	3269	12.2	7.40
		25	5260	15.3	7.42

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