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Advanced Engineering Informatics

Elixir Adv. Engg. Info. 42 (2012) 6335-6339

ISSN: 2229-712X

Bioreactor studies on biodegradation of phenol by Microaerophilic bacterial consortia

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ARTICLE INFO

Article history:

Received: 27 October 2011;

Received in revised form:

5 January 2012;

Accepted: 18 January 2012;

Keywords

Phenolic compounds,

Biodegradation,

Micro aerophilic bacteria.

ABSTRACT

Environmental pollution has been a major irritant to industrial development. Chemical and chemical based industries are prime targets of the environmentalists for their crusade against pollution. Leather industry contributes to one of the major industrial pollution with the generation of pollutants causing chemicals namely lime, sodium sulphide, salts, organic solvents and phenolic compound. Leather industry has faced serious challenges on phenolic compounds. Thus this work focused on evaluation of biodegradation of phenol by using microaerophilic bacterial consortium to overcome the deleterious effect of chemical effluents to the environment. This biodegradation process will be ecofriendly and cost effective. This could be one of the ways of solving the industrial pollution resulting from tannery effluent. Tannery effluent with high phenol level fed in both batch reactor and continuous reactor by using sustainable Microaerophilic Bacterial consortia to degrade the phenolic compounds.

Introduction

Environmental impacts associated with tanning and leather finishing primarily is related to the high organic load in the process effluents and the use of hazardous chemicals. Potential impact sources include Wastewater, Emissions in air, Solid waste, Hazardous materials. The quantities and qualities of emissions and waste produced by tanneries strongly depend on the type of leather processed, the source of hides and skins, and the techniques applied. A significant amount and variety of chemicals and proprietary products are used in the processes.

Leather Production Technology

Leather production consists of four processes. These are: a) Beam house operations in which salt, dirt and hair are removed by desalting and soaking, unhairing and liming, deliming and bating. b) Tan yard operations which involve Chrome tanning or Vegetable tanning that makes the hide resistant to bacteria and high temperature. c) Post-tanning operations (wet finishing) includes neutralization, retanning, dying and fat liquoring. The pollutants from the process include chrome, salt, dyestuff residues, fat liquoring agents and vegetable tannins are removed. d) Finishing operations is the end process in which the leather is given desired properties by removing the major pollutants and emissions. . Also, alkaline sulphide may be converted to hydrogen sulphide if the pH is less than 8.0 with the removal of COD to oxidize organic waste.

Pollution load in leather processing

About 75 percent of the organic load measured as chemical oxygen demand (COD) is produced in the beam house, with the main contribution coming from liming/dehairing processes through hair dissolving. Dehairing is also the main generator of total suspended solids. Another important source of COD is the degreasing process. Total COD concentrations can reach 200,000 mg/l. The following measures should be taken to reduce the organic load of these wastewater streams:

- Screen wastewater to remove large solids;
- Use an enzymatic dehairing process and recover hair for resale (reducing COD up to 40–50 percent)
- If conventional lime dehairing process is used, filter wastewater to recover hair before dissolution (typically reducing COD by 15–20 percent and total nitrogen by 25– 30 percent in mixed tannery effluent)
- Recycle liming float, which not only reduces COD by 30–40 percent and nitrogen by up to 35 percent, but also reduces the use of sulfide (up to 40 percent) and lime (up to 50 percent)
- Use easily degraded ethoxylated fatty alcohols as surfactants in degreasing to replace ethoxylated alkylphenols
- Use carbon dioxide (CO2) deliming (e.g., for light bovine hides (i.e., thickness < 3 mm)). For thicker hides, the process is slow and it is necessary to increase the float temperature (up to 35°C), and/or process duration and/or to add small amounts of deliming auxiliaries.

Signifance of Bacterial Consortium

Biodegradation processes using specific bacteria, to destroy toxic waste offer many advantages over physiological processes. When successfully operated, they are non polluting because such bacteria can completely degrade and oxidize toxic organic compounds to innocuous carbon compounds; are characterized by low costs; and offer the possibility of in situ treatment. Bioaugmentation is a process in which specific bacteria cultures are seeded in the bioreactor with ability to degrade the organic compounds contributing to COD and BOD values. The applications of specific bacteria developed for the treatment of effluents containing aromatic and aliphatic organic compounds from various industries and their successful implementation at plant scale level.

Biological Treatment Process

A common set of processes that might be found at a municipal treatment plant would be:

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- **Preliminary treatment**

To remove large or hard solids that might clog or damage other equipment. These might include grinders, bar screens, and grit channels. The first chops up rags and trash; the second simply catches large objects, which can be raked off; the third allows heavier materials, like sand and stones, to settle out, so that they will not cause abrasive wear on downstream equipment. Grit channels also remove larger food particles

- **Primary settling basins,**

The water flows slowly for up to a few hours, to allow organic suspended matter to settle out or float to the surface. Most of this material has a density not much different from that of water, so it needs to be given enough time to separate. Settling tanks can be rectangular or circular. In either type, the tank needs to be designed with some type of scrapers at the bottom to collect the settled sludge and direct it to a pit from which it can be pumped for further treatment-- and skimmers at the surface, to collect the material that floats to the top (which is given the rather inglorious name of "scum".) The diagram below shows the operation of a typical primary settling tank.

- **Secondary treatment**

Usually biological, tries to remove the remaining dissolved or colloidal organic matter. Generally, the biodegradation of the pollutants is allowed to take place in a location where plenty of air can be supplied to the microorganisms. This promotes formation of the less offensive, oxidized products. There are two major types of biological treatment processes: attached growth and suspended growth. In an attached growth process, the microorganisms grow on a surface, such as rock or plastic. Examples are Open trickling filters, enclosed biotowers, Rotating biological contacters (RBC's).

Activated sludge system.

This type of system consists of two parts, an aeration tank and a settling tank, or clarifier. The aeration tank contains a "sludge" which is what could be best described as a "mixed microbial culture", containing mostly bacteria, as well as protozoa, fungi, algae, etc. This sludge is constantly mixed and aerated either by compressed air bubblers located along the bottom, or by mechanical aerators on the surface. The wastewater to be treated enters the tank and mixes with the culture, which uses the organic compounds for growth--producing more microorganisms-- and for respiration, which results mostly in the formation of carbon dioxide and water. The process can also be set up to provide biological removal of the nutrients nitrogen and phosphorus.

Operating condition for bioreactor:

The bioreactor was thoroughly cleaned and autoclaved together with synthetic medium. The bioreactor was maintained in a continuous operation with an 150 rpm, at the optimum pH of 7.00 and at temperature of 30^{o}C with DO 1 ppm. The working volume in the bioreactor was maintained at 2.5 liters to provide a sufficient headspace to prevent air clogging in the funnel membranes provided. Online parameters were recorded daily for RPM, temperature, pH, and dissolved oxygen.

Phenol Analysis by Photometric Method

100ml distillate or a portion containing not more than 0.5g phenol diluted to 100ml is placed in a 250ml beaker. 100ml distilled water blank and a series of 100ml phenol standards containing 0.1,0.2,0.3,0.4,0.5 mg phenol are prepared. 2.5ml 0.5N ammonium hydroxide solution is added and pH is maintained at 7.9±0.1with phosphate buffer. 1.0ml 4-aminoantipyrine solution is added and mixed well. 1.0ml potassium ferricyanide solution is added and mix ed well. After 15 mins, transfered to cells and absorbance of sample and standards against the blank are read at 510nm.

Phenol Analysis by Standard Titration Method

Different concentration of sample are taken in a separate conical flask. The entire are made upto 100ml distilled water.10ml of bromide water and 5ml of concentrated HCL are added. Color changes to reddish brown color. (Note:If no color changes, add bromide water till it reach reddish brown color.)Then it is kept in a dark place for 10mins. one drop of potassium iodine is titrated with thio solution. Yellow color is reached. after that starch indicator is added and the solution became dark blue.then Titrated with thio solution, till it become colorless.

Chemical Oxygen Demand:

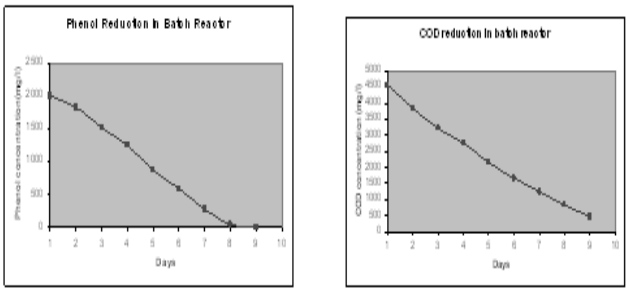
50.0ml sample is placed in a 500ml refluxing flask. 1g mercuric sulphate is added and several glass beads are added very slowly. 5.0ml of sulphuric acid is added with mixing to dissolve mercuric sulphate. cooled while mixing to avoid possible loss of volatile materials. 25.0ml of 0.0417M potassium dichromate solution is added and mixed.Attached a flask to condenser and turned to cooling water. Remaining sulphuric acid is added through open end of condenser. Swirling and mixing are continued while adding the sulphuric acid agent. open end of condenser is covered with small beaker to prevent foreign material from entering refluxing mixture and refluxed for 2 hours. It is cooled and washed. Excess potassium dichromate is titrated with ferrous ammonium sulphate using 0.10 to 0.15 ml (2 to 3 drops) ferroin indicator. Take as the end point of the titration the first sharp color change from blue green to reddish brown that persists for one min or longer. Duplicate determinations should agree within 5 % their average. Samples with suspended solids or components that are slow to oxidize may appear. In the same manner, reflux and titrate a blank containing the reagent and a volume of distilled water equal to that of samples.

Result and Discussion

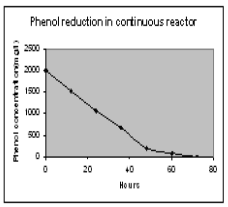
Performance efficiency of reactor studies was conducted for synthetic medium containing 2000 mg/l phenol with a Sustainable Microaerophilic bacterial Consortium. The optimum conditions of pH 7, Dissolved oxygen 1 ppm, Temperature 30^{o}c, and 150 rpm was maintained for the reactor.

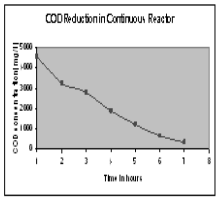
Phenol Degradation in Batch Reactor-Graph

In batch reactor process the level of phenol concentration reduced from 2000mg/l to 99.9% within 9th day. In batch reactor process, percentage of COD removal found to be 89.99 observed in 8th day. Similarly in continuous reactor process 2000mg/l was reduced gradually and 99.9% degradation was observed on 72th hour of process ,i.e. on 3rd day

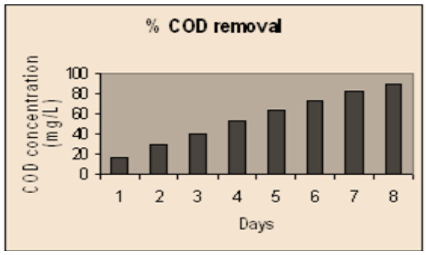


Phenol and COD reduction in batch reactor-graph

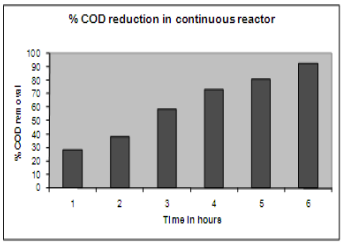




Phenol and COD Degradation in Continuous Reactor



Batch reactor process, percentage of COD removal found to be 89.99 observed in 8th day



Continuous process of reactor COD was reduced to 92.29% in 72nd hour of process. i.e. on 3rd day

From the above tables and graphs clearly indicate that continuous reactor performance is better than batch reactor, since the conditions were maintained at optimum throughout in the case of continuous reactor the performance were at the best in these when compared to batch studies. The SMB consortium is proved to be robust in native by its capacity to degrade phenol in 3 days.

The toxic pollutant, phenol present in tannery effluent almost removed through the bioprocess methods of continuous and batch reactor using sustainable microaerophilic bacterial consortium with maximum of 25 bacterial species.

Conclusion

To conclude from the above studies carried out the following points may be put forth. The given inoculum (SMB consortium) is capable of reducing COD and phenol.In continuous reactor where optimum condition was maintained through out the process is found to be better than the batch reactor. The SMB consortium can be exploited as an commercial tool in leather industrial treatment of waste water. The tannery liquid and solid wastes can be effectively treated through bioprocess by continuous and batch reactors and degrade the toxic pollutant phenol and make the environment clean without soil and water contamination. Leather industry contributes to one of the major industrial pollution with the generation of pollution causing chemicals namely lime, sodium, salts, sulphate and phenolic compound. Among this phenol is more toxic pollutant causing environmental pollution. Tannery effluent with high phenol level fed in both batch reactor and continuous reactor by using sustainable Microaerophilic Bacterial consortia to degrade the phenolic compounds contributing the COD values. Performance efficiency of batch and continuous reactor for synthetic medium containing 2000mg/l phenol with SMB consortia.

In batch reactor the level of phenol concentration reduced to 99.9% within 9th day. In continuous reactor the level of phenol concentration reduced to 99.9% in 72nd hours. Continuous is better over batch reactor. The SMB consortium is proved to be robust in native by its capacity to degrade phenol in 3 days.

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Composition of synthetic medium:

Phenol	1.0g
Dipotassium hydrogen phosphate	1.0g
Ammonium nitrate	1.0g
Ammonium sulphate	0.5g
Magnesium sulphate	0.5g
Potassium di hydrogen phosphate	0.5g
Sodium chloride	0.5g
Calcium chloride	0.02g
Ferrous sulphate	0.02g
Wolfe's mineral solution	10.0ml
Sodium thio glycolate	0.1

Sodium thio glycolate was added to culture media to maintain dissolved oxygen of not more than 2 mg/l

S.NO	Days	Phenol concentration (mg/l)
1	0	2000
2	1	1823
3	2	1512
4	3	1259
5	4	875
6	5	597
7	6	286
8	7	56
9	8	2

Phenol Reduction in Batch Reactor

S.No	Days	COD Concentration (mg/l)
1	0	4567
2	1	3831
3	2	3215
4	3	2758
5	4	2156
6	5	1653
7	6	1248
8	7	822
9	8	459

Cod Reduction In Batch Reactor

S.No	Time in hours	Phenol concentration (mg/l)
1	0	2000
2	12	1523
3	24	1076
4	36	676
5	48	194
6	60	87
7	72	2

Phenol Reduction In Continuous Reactor:

S.No	Hours	COD Concentration (mg/l)
1	0	4567
2	12	3289
3	24	2825
4	36	1904
5	48	1222
6	60	678
7	72	352

COD Reduction In Continuous Reactor:

S.No	Days	% COD removal
1	1	16.11
2	2	29.60
3	3	39.5
4	4	52.79
5	5	63.80
6	6	72.67
7	7	82.00
8	8	89.99

Percentage Cod Removal In Batch Reactor

S.No	Hours	% COD removal
1	12	27.98
2	24	38.14
3	36	58.3
4	48	73.24
5	60	80.77
6	72	92.29

Percentage Cod Removal In Continuous Reactor