# The Biodegradation of Brewery Wastes in a Two-Stage Immobilized System

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*Received: 28 January, 2002*
*Accepted: 8 April, 2002*

## Abstract

The investigations were made in a loop bioreactor, where biomass was immobilized in the ceramic carrier. The influence of the internal circulation rate on the biodegradation efficiency of brewery wastes by immobilized biomass and on production of surplus sludge was examined. The rates of the internal circulation were 12, 38, 50 $dm^3 \cdot h^{-1}$. The experiments were performed at constant loading rate of the carrier of 17.9 $g \cdot dm^{-3} \cdot h^{-1}$ and hydraulic retention time of 3 h. Increasing in the internal circulation rate from 12 to 50 $dm^3 \cdot h^{-1}$ caused enhancement of the removal rate from 0.40 to 0.48 $gCOD \cdot dm^3 \cdot h^{-1}$ and limitation of surplus sludge productivity from 0.67 to 0.27 $g \cdot g^{-1}$ COD removed.

The biodegradation rate of brewery wastes in a two-stage immobilized system was determined. The hydraulic retention time in this two-stage immobilized system was 6 h, which was enough to get a COD below 150 $mg \cdot dm^{-3}$ in the effluent.

**Keywords!** ceramic carrier, silicon carbide carrier, loop reactor, immobilization, brewery wastes

## Introduction

Much effort has been done to intensify wastewater treatment processes by increasing concentration of biomass in a reactor. Because of retaining great concentrations of sludge biomass it is possible to treat high volumetric organic loading [1]. For this reason using high-strength microorganisms has big advantages over traditionally activated sludge processes. One of the possibilities is the use of immobilization methods. Whole cell immobilization methods can now be divided into five major categories based on the physical attribute responsible for immobilization: surface attachment to matrices, entrapment in porous or polymeric matrices, encapsulation, containment behind a barrier, and self aggregation by flocculation.

Currently it is generally considered that colonisation of inorganic supports and attachments to modified surfaces are more suitable for a large-scale operation than entrapment in natural polymers. Colonisation of inorganic carriers by biomass is actually one of the most widely applied methods for the immobilization of active microorganisms. The multi-channel carrier used in this work presents different advantages. Silicon carbide is an inert material and shows high mechanical and chemical resistance. It has an ideal porous shape allowing high biomass load and minimal mass transfer problems. Mechanical stability of the carrier, biomass hold-up and hydraulic characteristic in the reactor make it extremely suitable for practical application in wastewater treatment.

## Experimental Procedures

### Multi-Channel Loop Reactor

The multi-channel ceramic carrier with immobilized biomass made the stationary filling of the reactor. The car-

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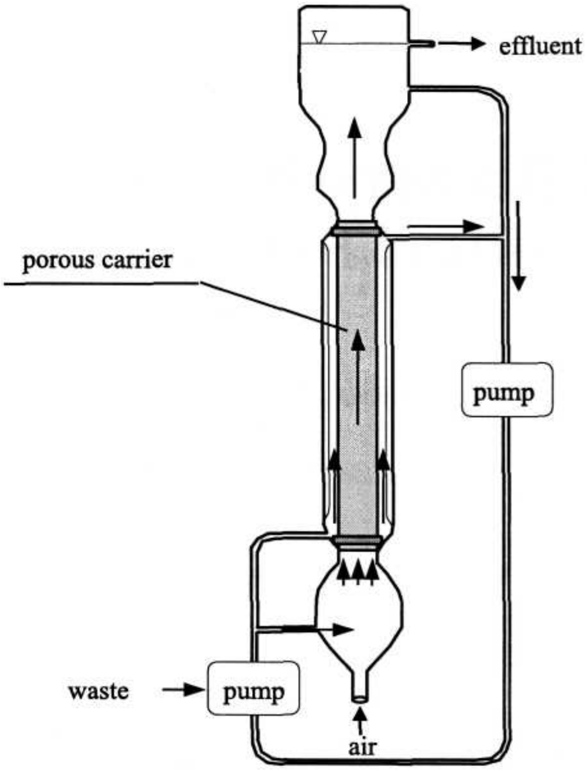


Fig. 1. Scheme of reactor.

rier was a silicon carbide matrix of the following characteristics: length 900 mm, external diameter 25 mm, number of internal channels 37, channel diameter 2 mm, void volume 60% of support volume, pore size from 8 to 100 μm.

The reactor design is shown in Fig. 1. The multi-channel carrier was fastened at both ends with O-rings in a glass cylinder. Two spaces were created: the internal channels and the external space. Raw wastewater flux and the circulating stream were mixed before they flew into the reactor. The influent was divided into two streams flowing parallel through the external space and internal channels. It allowed us to keep equal pressure on the internal and external surfaces. In this configuration the liquid circulation through the internal channels was in the range 12 to 50 $dm^3 \cdot h^{-1}$. The bioreactor worked under aerobic conditions. Air was taken to the carrier from below.

## Colonization Procedure

Immobilization of the activated sludge in the ceramic carrier was made by circulation of the activated sludge of an initial concentration 19 g dry mass$\cdot dm^{-3}$ into the bioreactor for 24 h. After completion of the colonization step the concentration of biomass in the circulation effluent dropped to 6 g dry mass$\cdot dm^{-3}$. In order to stabilize the composition of the biomass immobilized in the carrier two runs of circulating brewing wastes, lasting 24 hours each, were performed in the loop reactor at the rate of 50 $dm^3 \cdot h^{-1}$.

## Experimental Design

The wastes used in the experiments were a mixture of the effluents taken from the brewing and fermentation departments of one Belgian brewery. Organic compound content of this brewery waste is presented in Table 1.

The experiments were done in two series. In the first series the influence of the internal circulation rate on the reaction rate and productivity of surplus sludge were examined. The rates of internal circulation were 12, 38, 50 $dm^3 \cdot h^{-1}$. The experiments were performed at the constant loading rate of the carrier with immobilized biomass equal to 17.9 $g \cdot dm^{-3} \cdot d^{-1}$ and hydraulic retention time of 3 h.

Table 1. Average concentration of organic compounds in the investigated brewery wastes.

| Organic compounds | Unit | Value |
|---|---|---|
| COD | $mg \cdot dm^{-3}$ | 2200 |
| Carbohydrates and organic acids | $mg \cdot dm^{-3}$ | 57 |
| Lactic acid | $mg \cdot dm^{-3}$ | 35 |
| Acetic acid | $mg \cdot dm^{-3}$ | 0.5 |

In the second series the biodegradation rate of brewery wastes in two coupled stages of the immobilized system was determined. The experiments in the second series were conducted at the constant organic load rate in stage I and at two different organic load rates in the second stage of the system. Technological parameters are presented in Table 2.

Table 2. The technological parameters of two stages immobilized system.

| Parameter | Unit | Stage I | Stage II run 1 | Stage II run 2 |
|---|---|---|---|---|
| Volume of carrier with immobilized biomass | $dm^3$ | 0.89 | 0.89 | 0.89 |
| Flow rate of wastes | $dm^3 \cdot h^{-1}$ | 0.295 | 0.295 | 0.770 |
| Internal circulation rate | $dm^3 \cdot h^{-1}$ | 50 | 50 | 50 |
| Dilution rate (D) | $h^{-1}$ | 0.018 | 0.018 | 0.017 |
| Organic load | $g \cdot d^{-1}$ | 15.6 | 5.1 | 13.4 |

## Analytical Methods

The chemical oxygen demand was determined colorimetrically using COD digestion reagent vials (Hach Co.). Total suspended solids (TSS) were determined by the methods described in Standard Methods [2].

Organic acids concentration in medium was determined with a Shodex Ionpak KC-810P 5 cm pre-column and two Shodex-Ionpak KC 811 30 cm columns, using Perkin-Elmer, Series 3B Liquid Chromatograph equipped with Waters 410 Differential Refractometer and C-R5A Chromatopack Shimadzu integrator. Solvent used: 0.1% $HClO_4$.

Concentration of volatile compounds in brewery wastes influent and effluent was made by head space method Chromatographe Perkin-Elmer 8500, with a WCOT FS 50 m0.32 mm Df 1.2 CP WAX-52 CB column.

Concentration of carbohydrates in medium was determined with Waters Sugar-Pak I column using a Perkin-Elmer, Series 10 Liquid Chromatograph equipped with Waters 410 Differential Refractometer and C-R6A Chromatopack Shimadzu integrator. Solvent used: water (>2 MOhm resistance) and 50 $mg \cdot dm^{-3}$ CaEDTA.

## Results

The results indicated that during brewery wastes biodegradation by biomass immobilized in ceramic carrier an increase in the internal circulation rate from 12 to 50 $dm^3 \cdot h^{-1}$ caused enhancement of the removal rate from 0.40 to 0.48 $gCOD \cdot dm^{-3} \cdot h^{-1}$ and surplus sludge production drop from 0.67 to 0.27 $g \cdot g^{-1}$ COD removed (Tab. 3).

Table 3. The effect of internal circulation rate on removal rate and surplus sludge production in the immobilized system (mean values).

| Internal circulation rate $dm^3 \cdot h^{-1}$ | Dilution rate (D) $h^{-1}$ | Removal rate g $COD \cdot dm^3 \cdot h^{-1}$ | Production of surplus sludge $g \cdot g^{-1}$ COD* |
|---|---|---|---|
| 50 | 0.018 | 0.48 | 0.27 |
| 38 | 0.022 | 0.45 | 0.30 |
| 12 | 0.075 | 0.40 | 0.67 |

*removed COD

At the internal circulation rate of 50 $dm^3 \cdot h^{-1}$ the immobilized system was exploited at the lowest values of the dilution rate and this presumably caused a drop in surplus sludge productivity resulting from biomass synthesis.

The results obtained in the second series of the experiments are presented in Table 4.

Table 4. The efficiency of COD removal from brewery wastes in a two-stage immobilized system.

| Parameter | Unit | Stage | | |
|---|---|---|---|---|
| | | I | II run 1 | II run 2 |
| Influent COD | $mg \cdot dm^{-3}$ | 2200 | 726 | 726 |
| Effluent COD | $mg \cdot dm^{-3}$ | 726 | 110 | 185 |
| Removed COD | $mg \cdot dm^{-3}$ | 1474 | 616 | 541 |
| Organic load rate | $g \cdot dm^{-3} \cdot d^{-1}$ | 17.9 | 5.8 | 15.2 |
| COD removal | % | 67.0 | 84.8 | 74.5 |
| Total COD removal | % | | 94 | 90 |

In stage I of the immobilized system at the organic load rate of 17.9 $g \cdot dm^{-3} \cdot d^{-1}$ the efficiency of organic substance removal expressed as COD was 67%. In Table 5 the amounts of organic acids in brewery wastes in the influent and effluent of the system are presented.

In the effluent from stage I of the immobilized system the concentration of lactic acid of 32 $mg \cdot dm^{-3}$ was equal to that in the influent. During brewery waste biodegradation in stage I of the immobilized system the concentration of acetic acid grew up from 0.5 $mg \cdot dm^{-3}$ in the influent to 85 $mg \cdot dm^{-3}$ in the effluent. Also, small quantities of tricarboxylic acids (TCA) appeared, which had come from Krebs cycle in the effluent (data not shown).

After the biodegradation in stage I the wastes was pumped to stage II of the immobilized system. Stage II was run at the organic load rate of 5.8 $g \cdot dm^{-3} \cdot d^{-1}$ (Tab. 4, run 1). The obtained efficiency of COD removal was 85%. The effluent from stage II did not contain both lactic and acetic acids. In the effluent only small quantities of pyruvate and succinate were found.

Enhancement of the organic load rate in stage II from 5.8 to 15.2 $g \cdot dm^{-3} \cdot d^{-1}$ (Tab. 4, run 2), as a result of the hydraulic retention time shortening from 3 to 1.2 h, caused a decrease in the COD removal efficiency

Table 5. The average concentration of lactic and acetic acid in the brewery wastes in the influent and effluent of two stages immobilized system.

| Organic acid $mg \cdot dm^{-3}$ | Stage I | | Stage II (run 1) | | Stage II (run 2) | |
|---|---|---|---|---|---|---|
| | influent | effluent | influent | effluent | influent | effluent |
| Lactic acid | 32 | 32 | 32 | no detectable | 32 | no detectable |
| Acetic acid | 0.5 | 85 | 85 | no detectable | 85 | no detectable |

to 74.5%. The observed decrease did not result from lower biodegradation efficiency. As in the previous run the effluent from the stage II did not contain lactic and acetic acids. The concentration of pyruvate and succinate was also low. The COD increase in the effluent from stage II of the immobilized system resulted from an increase in suspended solids quantity in the effluent (Tab. 6).

Table 6. The amount of suspended solids in the effluent and biomass yield in a two-stage immobilized system.

| Parameter | Unit | Stage | | |
|---|---|---|---|---|
| | | I | II run 1 | II run 2 |
| Suspended solids in effluent | $mg \cdot dm^{-3}$ | 440 | 12 | 150 |
| Biomass yield | $g \cdot g^{-1}COD^{*} \cdot d^{-1}$ | 0.290 | 0.016 | 0.280 |

* removed COD

It should be emphasised that the efficiencies of COD removal were obtained at limited growth of biomass (Tab. 6). The obtained results indicate a possibility of designing a two-stage immobilized system for brewery waste biodegradation with low surplus sludge productivity resulting from proper selection of the organic load rate. However, these results do not give definite answers concerning how long a two-stages immobilized system can be effective under the conditions of limited biomass growth.

## Discussion

A number of breweries are already running various types of the anaerobic plants for their wastewater pretreatment. In UASB-type reactors, the pre-acidified wastewater flows up from 0.75-1 $m \cdot h^{-1}$, resulting in a retention time of 4-6 hours and it is sufficient to obtain full conversion of acetate to methane and carbon dioxide. The organic load from 25 to 30 kg $COD \cdot m^{-3} \cdot d^{-1}$ is about maximum. As a result the effluent COD fluctuates around 500 $mg \cdot dm^{-3}$. Such effluent does not meet discharge standards. The post treatment should take place in the aerobic waste treatment plant to get effluent discharge equal to 150 $mg \cdot dm^{-3}$. The productivity of anaerobic surplus sludge is low, about 3 % of the COD removed. Other investigations [3] present the treatment of brewery wastewater by the ADUF$^R$ method (Anaerobic Digestion - Ultrafiltration). Those processes cause 96-99% of the COD reduction at the space load rate of 15 $kg \cdot m^{-3} \cdot d^{-1}$ and the hydraulic residence time of 12-19 hours. In the presented two-stage immobilized system the pre-acidified step was not needed. The two-stage immobilized system was operated in order to make the biodegradation efficiency of strong wastewater higher. In this system the organic load rate in stage I was 17.9 $g \cdot dm^{-3} \cdot d^{-1}$ and in stage II about 5.8 $g \cdot dm^{-3} \cdot d^{-1}$ (Tab. 4). The hydraulic retention time was 6 h. This was enough to get the effluent COD below 150 $mg \cdot dm^{-3}$. When the second stage was operated at the organic load rate about 5.8 $g \cdot dm^{-3} \cdot d^{-1}$ the surplus sludge productivity was low and equal to 1.7% of the COD removed. Although the reactor was operated at a high organic load rate and short hydraulic retention time, the big efficiency of carbon removal was received. This was caused by high concentration of microorganisms in the carrier. In the reactors with immobilized biomass the concentration of microorganisms may achieve over 30 $kg \cdot m^{-3}$, when the strength of biomass in the suspended activated sludge systems is 3-5 $kg \cdot m^{-3}$ [4]. According to Yamamoto at al. [5], the critical concentration of immobilized biomass, which allows stable filtration, is 30-40 $kg \cdot m^{-3}$. At high volumetric loading it is possible to obtain great biodegradation efficiency because the microbial solid retention time is longer than hydraulic retention time [3]. Besides, high effluent quality is possible because of the retention of colloidal and macromolecular material, including bacteria [6].

During brewery wastewater biodegradation it was explored that an increase in internal circulation rate caused increase in the degradation efficiency at low surplus sludge production. An increase in the internal circulation rate caused hydraulic loading rise. Big hydraulic loading did not cause immobilized biomass washout from the reactor. It was observed that the surplus sludge amount depended on changes in hydraulic loading. In the suspended activated sludge 2/3 of organic compounds are used for biomass synthesis and 1/3 for endogenous reactions. This caused the formation of large amounts of the excessive sludge. In the immobilized biomass systems cellular oxidation prevails probably over biomass synthesis. Canales et al. and Chiemchaisri et al. [7, 8] proved that high concentrations of biomass in the carrier causes low rates between substrate quantity and amount of microorganisms. Also, because of this phenomena the volume of surplus sludge is low.

Stars et al. [9] have observed that the accumulation of lactate occurred in the wastes as a response to low oxygen availability. In our investigations on stage I available carbon concentration was very high, and it is difficult to determine why the lactic acid was not degraded under such conditions. At the high loading of stage I some bacteria produced the volatile acids when oxygen was supplied.

## Conclusions

- Using ceramic carriers with immobilized biomass permits to get 94% of the carbon substrate elimination from brewery wastes. It was found that such elimination can be obtained at the following technological parameters: the organic load rate in stage I - 17.9 $g \cdot dm^{-3} \cdot d^{-1}$, and in stage II - 5.8 $g \cdot dm^{-3} \cdot d^{-1}$, the total retention time in two stages immobilized system - 6 h. Under the aerobic conditions it can be possible to get low biomass yield.
- In a multi-channel loop reactor more efficient supply of the substrate to immobilized cells in the pore space can be expected when the rate of internal circulation increases from 12 to 50 $dm^3 \cdot h^{-1}$.

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