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Biodegradation of VOCs from printing press air by an on-site pilot plant bioscrubber and laboratory scale continuous yeast cultures

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Abstract

The volatile organic compound composition (VOCs) of printing press air was found to contain mostly ethanol, but also ethyl acetate, 1-propanol, 2-propanol, 1-methoxy-2-propanol and 3-ethoxy-1-propanol. A pilot plant bioscrubber inoculated with a mixed microbial population was constructed on-site. The bioscrubber was able to treat the polluted gas efficiently. It, however, suffered from strong wall growth and blockages in the column. The efficiencies of the pilot plant and a bioreactor is compared. The yeasts *Candida guilliermondii* and *Saccharomyces cerevisiae* known to tolerate ethanol were selected instead of mixed population to avoid the wall growth and blockages in the bioreactor. The removal of the VOCs both individually and as a complex mixture was tested in a microcultivation system and in continuous chemostat cultures with and without cell recycling. The *Candida* yeast could use all the compounds as a carbon source while growth of S. cerevisiae was markedly slower on the methoxylated and ethoxylated propanols. Best total removal of the VOCs was 99% and achieved by *C. guilliermondii*. The only compound that was not totally removable in the chemostat experiment with *C. guilliermondii* was 1-methoxy-2-propanol. In laboratory scale the total and volumetric removal of VOCs by *C. guilliermondii* was more efficient compared to the pilot plant encouraging to scale up and applying the yeast bioreactor to real field conditions.

Introduction

In order to apply the bioprocess approach to removing VOCs three major constructions have been considered, biofilter, trickling biofilter and bioscrubber (Ottengraf 1987). Wu et al. (1998) constructed a biofilter for waste gases containing VOCs using the cultures of *Bacillus*, *Micrococcus*, *Acinetobacter* and yeast. They studied the biofiltration of 1-nitropropane and the mixture of iso-propane, acetate and 1-nitropropane. Barshter et al. (1993) presents case studies for removal of EtOH (<300 ppm) from waste gases of a yeast plant and pentane (600–800 ppm) from a chemical plant by biofiltration. Kellner et al. (1998) studied bioscrubber elimination of VOCs in coating machine waste gases, where acetone was the main pollutant. Loy et al. (1998) describe the problems with an industrial bioscrubber and a biotrickling plant with two case studies. Based on different laboratory and pilot tests, optimization of the installed bioscrubber is shown to give high efficiency in the elimination of VOCs. The second case study shows the problems of an industrial biotrickling plant. Daubert et al. (2001) used a gas/liquid contactor called and a membrane bioreactor in removing VOCs. They studied the transfer phenomena characterization using a gaseous effluent polluted with 7.1×10^{-3} kg/m^3 ethanol concentration. They obtained 90% absorption rate of ethanol in the liquid phase. *Candida utilis* was used as microorganism for ethanol elimination. No ethanol was measured in the fermentation broth nor in the outlet gas, confirming the efficiency of ethanol elimination. Bustard et al. (2000) studied the biodegradation of propanol and isopropanol by a mixed microbial population. Duetz et al. (1998) studied the biodegradation kinetics of toluene, *m*-xylene and *p*-xylene in a chemostat by genetically engin-

eered *Pseudomonas putida* harbouring TOL-plasmid (pWW0).

In the present study we have analysed the VOC content of the circulating air streams of a typical printing press operation. The main components were ethanol, 2-propanol, 1-propanol, ethyl acetate, 3-ethoxy-1-propanol and 1-methoxy-2-propanol. These compounds originated mainly from dye solvents used in printing. In modern printing operations the printing press is equipped with a burning device to minimise the evaporating pollutants. The pollutant problem is related to older printing presses and circulating air. We constructed an on-site pilot plant bioscrubber at the actual printing site, which was inoculated with a mixed microbial population acclimatised to degrade these volatile organic compounds. The use of mixed microbial population in pilot plant bioscrubber eventually gave rise to strong wall growth, slime formation and unpredictable oxygen consumption. Eventually, this blocked the air stream flow into the column and increased pressure in the bioreactor unit, which resulted in increased overflow. Due to these problems we concentrated our effort on improving the role of bioreactor unit and studied the continuous VOC removal characteristics in a laboratory scale chemostat. The yeasts *Saccharomyces cerevisiae* and *Candida guilliermondii* monocultures were selected on their ability to tolerate high ethanol concentrations. The effect of recycling was studied to scale down the constructed pilot plant bioscrubber.

Materials and methods

Organism, maintenance and inoculum preparation

Saccharomyces cerevisiae VTT-B-670002 and *Candida guilliermondii* VTT-C-71006 were obtained from VTT Biotechnology Research Laboratory (Espoo, Finland). Frozen stock cultures containing 20% (w/v) glycerol were stored in 2 ml ampoules at −70 °C. The inoculum for fermentation was prepared in 250-ml shake flasks grown overnight on YM-medium at 30 °C and 200 rpm. The YM-medium contained 3 g/l yeast extract (Difco), 3 g/l malt extract (Difco), 5 g/l bactopeptone (Difco) and 10 g/l glucose (Fluka). The bioreactor was inoculated with 100 ml of yeast suspension.

Construction of the on-site pilot plant

The bioscrubber was constructed and connected to the exhaust gas channel of the printing press. The volumes of the exchange column and the bioreactor unit were 35 L and 120 L, respectively (Figure 1). Glass beads were used as packing material for exchange column. The VOCs were absorbed into an aqueous phase (recirculating effluent), which was used as a substrate for the mixed microbial population. The fresh mineral supply rate (mineral medium without the VOCs) into the bioreactor unit ranged between 10–60 ml/min. The effluent leaving the bioreactor unit was recirculated through the exchange column with a flow rate ranging between 3.2–12 L/min. The dissolved oxygen level and pH was monitored continuously. Gaseous and liquid samples were taken to aid in designing the medium for chemostat cultivations and for further analysis. The mineral medium for on-site pilot contained per litre: NH_4NO_3 2.0 g, Na_2HPO_4 0.6 g, KH_2PO_4 1.5 g, $MgSO_4{\cdot}7H_2O$ 0.2 g, $CaCl_2{\cdot}2H_2O$ 0.01 g, EDTA 1.3 g, $ZnSO_4$ 68 mg, $CoCl{\cdot}6H_2O$ 24 mg, $MnCl_2{\cdot}4H_2O$ 99 mg, $CuCl_4{\cdot}2H_2O$ 17 mg, $FeCl_3{\cdot}4H_2O$ 2.0 g, $NaMoO_4{\cdot}2H_2O$ 24 mg, H_3BO_4 62 mg, $NiCl{\cdot}6H_2O$ 24 mg. An antifoam agent (Struktol J633) was added into the bioreactor unit, when necessary at 0.1–0.3 ml/L. The efficiency of VOCs removal of the bioscrubber column was measured with continuous FID analysator.

Chemostat experiments

The mineral medium for continuous culture experiments was prepared according to Verduyn et al. (1992) containing per litre: $(NH_4)_2SO_4$ 5.0 g, KH_2PO_4 3.0 g, $MgSO_4{\cdot}7H_2O$ 0.5 g, EDTA 15.0 mg, $ZnSO_4{\cdot}7H_2O$ 4.5 mg, $CoCl{\cdot}6H_2O$ 0.3 mg, $MnCl_2{\cdot}4H_2O$ 1.0 mg, $CuSO_4{\cdot}5H_2O$ 0.3 mg, $CaCl_2{\cdot}2H_2O$ 4.5 mg, $FeSO_4{\cdot}7H_2O$ 3.0 mg, $NaMoO_4{\cdot}2H_2O$ 0.4 mg, H_3BO_4 1.0 mg, KI 0.1 mg and silicon based antifoam agent 0.05 mg (BDH). The mineral medium was autoclaved for 20 min at 120 °C. After autoclaving a filter sterilised vitamin solution was added giving a final concentration per litre of biotin 0.05 mg, calcium panthotenate 1.0 mg, nicotinic acid 1.0 mg, *myo*-inositol 25 mg, pyridoxal hydrochloride 1.0 mg and para-aminobenzoic acid 0.2 mg. Volatile organic compounds (VOC) were added (based on exhaust gas analysis coming from the printing press) after the autoclavation, containing per litre: ethanol 6.0 g (Primalco), ethyl acetate 0.53 g (May-Baker), 1-propanol 0.16 g (Merck), 2-propanol 0.32 g (Riedel de Haën),

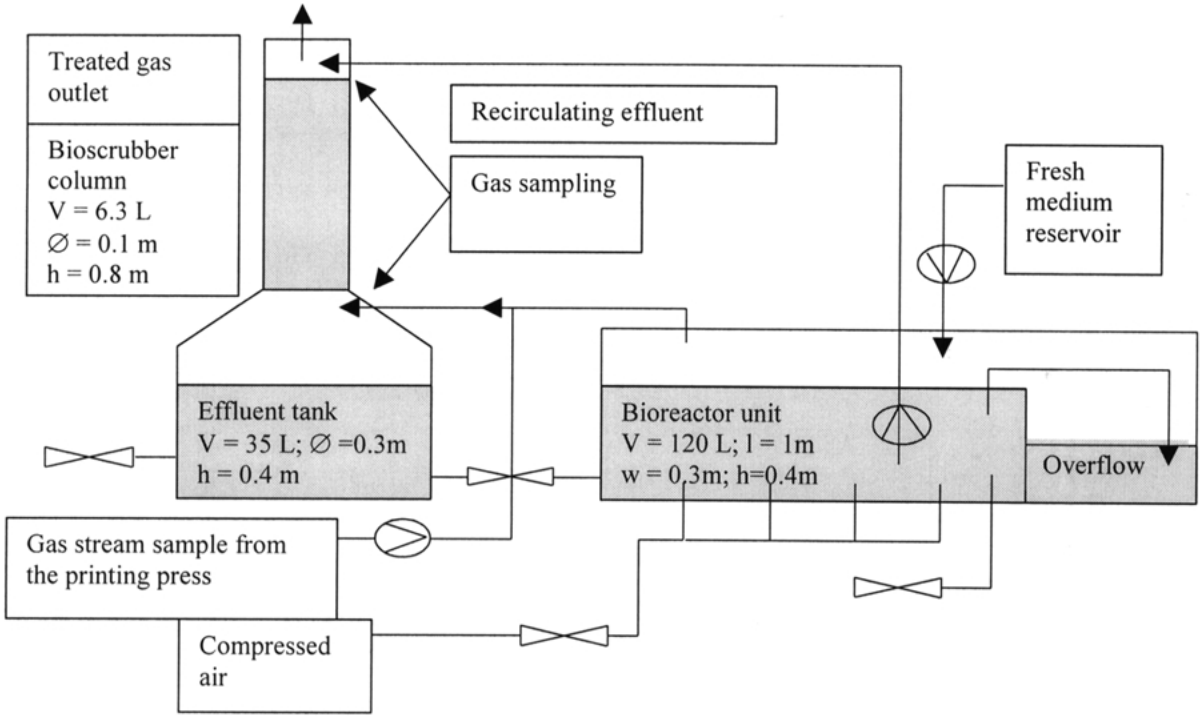

Figure 1. Schematic presentation of the pilot plant bioscrubber constructed on-site connected to the exhaust gas line of the printing press. Dissolved oxygen, pH and temperature were measured continuously. The highest exhaust gas stream rate channelled to the bioscrubber column was 3.72 m^3/h containing 12.5 g of VOCs.

3-ethoxy-1-propanol 0.62 g (Aldrich) and 1-methoxy-2-propanol 0.08 g (Aldrich). The final concentration of the medium was determined at the end of each chemostat run. The chemostat was always inoculated with single yeast culture at a time and culture purity was monitored on a regular basis by phase contrast microscopy.

Chemostat cultivations were carried out in a 2-litre fermenter (Braun MD) on a mineral medium at 30 °C with a stirrer speed of 1000 rpm (Figure 2). The culture pH was set at 5.0 and the dilution rate was adjusted to the desired value. The working volume of 1000 ml (the amount of yeast suspension in the bioreactor) was kept constant by removing the effluent with a peristaltic pump (Watson-Marlow 101U) that was connected to a PID-controlled load cell. The accurate working volume was measured at the end of each experiment. The culture pH was kept constant by addition of 2M KOH. The airflow rate was set to 0.5 L/min, which was sufficient to maintain the dissolved oxygen concentration above 30% in all aerobic cultivations. It was controlled using a mass flow controller (Bronkhurst HiTec, Ruurlo, Holland). The dissolved oxygen concentration was measured with an O_2-electrode (Ingold). The steady state was confirmed by measuring the biomass, metabolites and gas values on a two successive days. The unchanged values indicated that a steady state have been established. In all cases five working volume changes were sufficient to establish a steady state.

Recycling

The yeast cell suspension was recycled through a continuously working, sterilisable and magnetically stirred on-line recycling unit (BIOPEM, B. Braun Melsungen AG, Germany), which was connected by silicon tubes to the bioreactor (Figure 2). Retentate and permeate flows were controlled using peristaltic pumps (Watson-Marlow 101U). Retentate flow was set to recycle 254 ml/h but permeate flow was varied according to experiment. The permeate flow was filtered through a 0.22 μm polyvinylidene difluoride filter (Millipore) and weighed daily in order to determine the exact outlet flow rate. The working volume addition due to the recycling unit was 320 ml, which was taken into consideration when dilution rate was calculated.

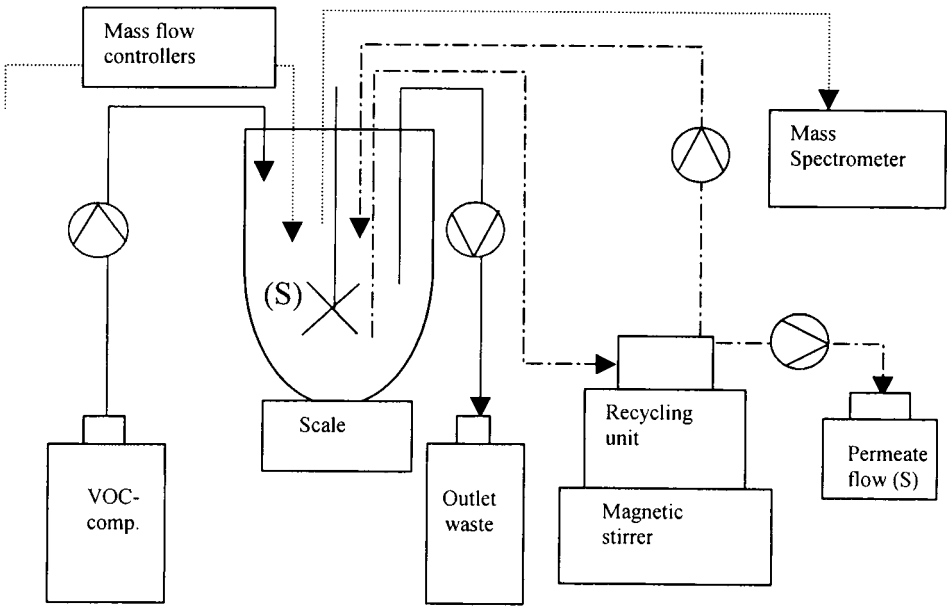

Figure 2. Schematic presentation of the chemostat set up and the recycling device. Samples were taken from the fermenter and the permeate flow (marked with S). The flow and recycling direction have been separated with dashed line and dashed dotted line.

Exhaust gas analysis

The fermentation exhaust gas was cooled to 4 °C in a condenser to prevent the evaporation of volatile compounds before entering the mass spectrometer (VG-Prima 600). Carbon dioxide and oxygen were analysed from exhaust gas. Nitrogen and argon not consumed by the yeasts were measured as an internal standard. In calculating the oxygen consumption rates and carbon dioxide production rates a temperature of 30 °C was assumed, and air pressure was taken from the daily weather forecast.

Cell dry weight measurements

Culture samples (10 ml) were vacuum filtered through preweighed nitro-cellulose filters (0.45 μm, Schleicher & Schuell), washed with Milli-Q water, and dried in a microwave oven for 20-min (Ignis, Japan). All the biomass determination from each steady state were done duplicates.

Substrate and metabolite analysis

Liquid sample volumes of 1.5 ml collected aseptically from the chemostat and from the permeate flow of the recyling unit were centrifuged at 8000 g for 5 min (Heraeus Sepatech, Biofuge A, Germany) and the supernatant was stored at −20 °C for further analysis. VOCs were analysed with gas chromatography (HP 6809), using an HP-INNOWAX column (30 m, 0.32 mm, 0.5 μm, USA) and flame ionisation detector (FID). The operating conditions were as follows: injector temperature 200 °C and detector temperature 240 °C. The initial temperature of the oven was 40 °C for 4 minutes, after that the temperature was increased with a rate of 10 °C/min until 110 °C, where it remained for 10 minutes. At the end of the run the temperature was raised to 165°C with a rate of 20 °C/min. Helium was used as a carrier gas, injection volume was 3 μl and the split ratio was 1:50. At the pilot plant gas samples were collected in sample bags and analysed correspondingly.

Results

A schematic presentation of the pilot plant bioscrubber constructed on-site connected to a printing press is presented in Figure 1. During the printing press operation the typical gas stream flow from the printing press was 6300 m^3/h and the gas stream temperature was between 50–60°C. The average exhaust gas flow rate channelled to the bioreactor unit ranged from 1.68–3.72 m^3/h and the temperature was 23 °C. The gas stream from the printing press was measured to

contain per m^3 of VOCs: 4.7 g ethanol, 0.7 g 3-ethoxy-1-propanol, 0.5 g ethyl acetate, 0.3 g 2-propanol, 0.1 g 1-propanol, 0.04 g 1-methoxy-2-propanol giving a total concentration of 3.4 g of carbon VOC/m^3. After the bioscrubber column the VOC concentration dropped down to a level of 20 mg of carbon VOC/m^3 being only 0.6% of the original carbon amount. The average dry weight of the pilot plant microbial suspension was 0.45 g/l. The maximum specific ethanol consumption rate varied between 0.1–0.4 g/g dwt/h. Ethanol and 3-ethoxy-1-propanol were found in the overflow. Their concentrations varied between 0–60 mg/L and 80–300 mg/L, respectively. The pH of the bioreactor unit was between 6–7 and the temperature varied from 19 to 25 °C. The oxygen concentration was frequently close to 0 mg/L, when exhaust gas with VOCs was channelled to the bioreactor. When purging the bioreactor unit with pure air stream the oxygen concentration increased approximately to 6 mg/L.

As a preliminary laboratory scale experiments, each of the VOCs were separately tested in the Bioscreen microcultivation apparatus as a sole carbon source for two different yeasts (results not shown). *C. guilliermondii* was able to grow on each of them individually and on a mixture. Growth of *S. cerevisiae* was approximately 50% slower with all of the VOCs compared to *C. guilliermondii* and particularly with methoxylated and ethoxylated-propanols. These results indicated that both of these yeasts can be used in the bioreactor experiments to remove VOCs. The VOC composition of the gas stream from the printing press was used to design a minimal carbon limited medium for continuous chemostat cultures. Altogether six steady state chemostat cultivations were carried out with or without cell recycling. The chemostat set up is presented in the Figure 2 and the basic physiological data of the cultivations and are given in Table 1. In addition, Table 2 represents the mean values of the inflows and outflows of the chemostat runs in order to indicate the repeatibility of the experimental set up. *C. guilliermondii* grew more effectively on the VOCs mixture, which can be seen in higher yields on biomass (runs 1 and 5 are directly comparable). The oxygen consumption and carbon dioxide production rates and RQ were higher with *S. cerevisiae* than with *C. guilliermondii*.

Specific consumption rates of the individual VOC compounds and total carbon consumption are given in Figure 3. In runs 1 and 5, which are comparable in respect to the dilution rate, the specific consumption rates of ethyl acetate, 2-propanol, ethanol and 1-propanol were higher for S. cerevisiae, consequently the total specific consumption rate was 75% higher than with *C. guilliermondii*. However, the consumption of 3-ethoxy-1-propanol and 1-methoxy-2-propanol was less effective with *S. cerevisiae* than with *C. guilliermondii* in all conditions. In *C. guilliermondii* higher dilution rate had a significant effect on the specific consumption rates of ethyl acetate, 2-propanol, ethanol, 1-propanol and 3-ethoxy-1-propanol, whereas the specific consumption of 1-methoxy-2-propanol remained essentially the same (runs 1 and 2). The total specific consumption rate of run 2 was 59% higher than in run 1. Recycling improved the total specific consumption rate of *C. guilliermondii* by 16% (runs 2 and 4), however the specific consumption rate of 3-ethoxy-1-propanol was decreased 10-fold. At the lower dilution rate (runs 1 and 3) the specific consumption rate of 3-ethoxy-1-propanol was increased 4.5-fold due to the recycling.

Figure 4 shows the percentage removal of the individual VOCs in the different runs. *S. cerevisiae* was not able to use any of the substrates completely. The highest total carbon removal of 77% was achieved with recycling. At the dilution rate of 0.1 h^{-1} the total carbon removal by *S. cerevisiae* was 51% without recycling (run 5). The highest total carbon removal of *C. guilliermondii* was 99% in run 3 with cell recycling. Both yeast strains could remove ethyl acetate, ethanol, 1-propanol, 2-propanol, but the most difficult compounds to remove were methoxylated- and ethoxylated-propanols. Cell recycling improved the removal of these compounds with *S. cerevisiae* moderately (runs 5 and 6). In case of *C. guilliermondii* cell recycling improved the removal of 3-ethoxy-1-propanol (runs 1 and 3), however this was strongly dependent on dilution rate (runs 2 and 4). The removal of 1-methoxy-2-propanol remained essentially the same and it was not improved either by cell recycling or by dilution rate. In the recycling experiments the VOC concentrations in the permeate were approximately the same as in the chemostat (results not shown). Permeate flow was not recycled back to the chemostat.

Discussion

The bioscrubber unit operated efficiently and the measured removal rate of the VOCs was 99.6% excluding the evaporation losses. However, the use of mixed microbial population in pilot plant bioscrubber

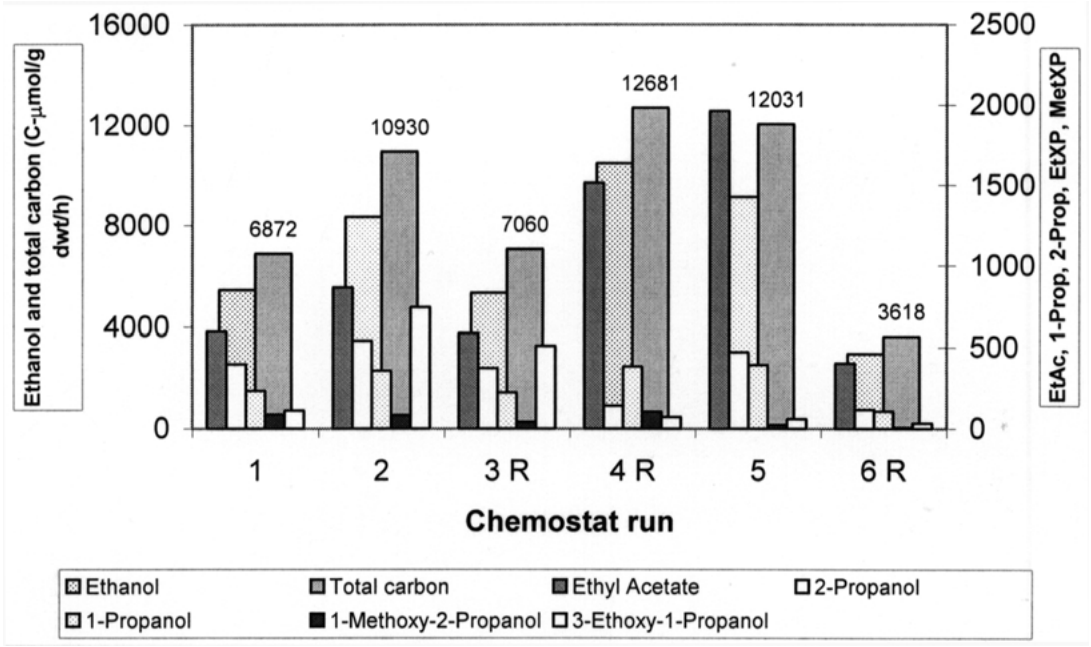

Figure 3. Specific carbon consumption rates of the VOCs in the chemostat by *C. guilliermondii* (runs 1–4) and *S. cerevisiae* (runs 5–6). The recycling unit was connected in the chemostat runs 3, 4 and 6 (marked with R). All the values are normalised according to the biomass. The numbers indicate the total carbon consumption (C-μmol/g dwt/h).

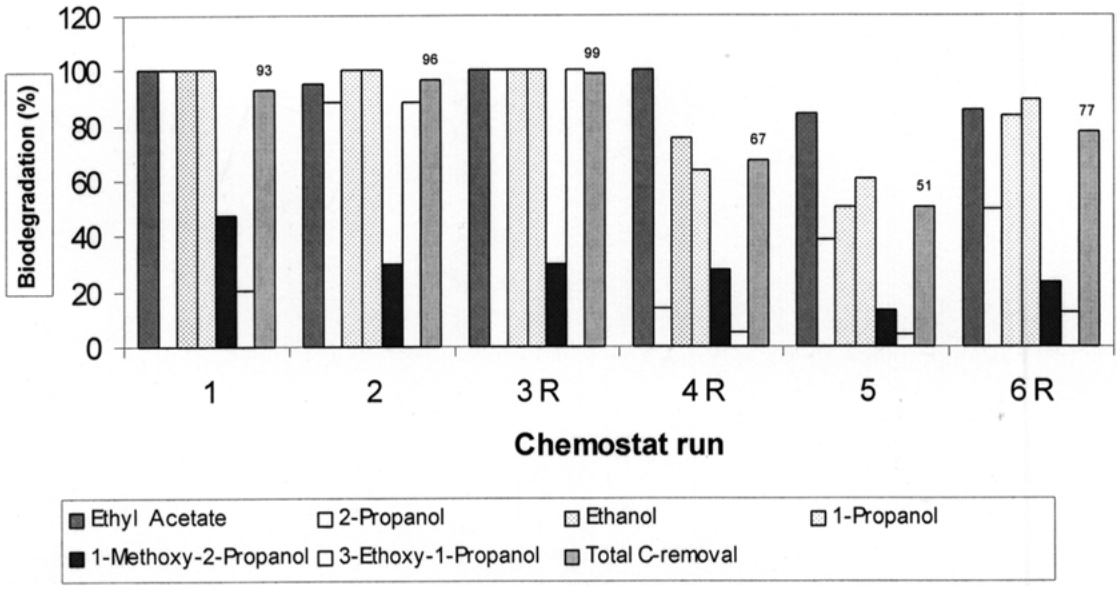

Figure 4. Removal of the individual VOC-compounds and total carbon removal in the chemostat. The total carbon is the sum of the individual VOC-compounds calculated as a carbon substrate. Runs 1–4 were carried out with *C. guilliermondii* and runs 5–6 with *S. cerevisiae*. In runs 3, 4 and 6 the recirculation unit was connected to the bioreactor (marked with R).

Table 1. Basic physiological data of the chemostat cultivations with *C. guilliermondii* (runs 1–4) and *S. cerevisiae* (runs 5 and 6). Chemostat runs were carried out at pH 5 and 30 °C in the mineral medium with the mixture of VOCs as a carbon substrate

Run	D h^{-1}	Recycle ml/h	Permeate ml/h	Biomass (g/l)	Yx/C-mol (g dwt/C-mol)	q(O2) mmol/g dwt/h	q(CO2) mmol/g dwt/h	RQ
1	0.100	no	no	4.78	14.5	4.28	1.85	0.43
2	0.141	no	no	4.43	12.9	6.21	2.88	0.46
3	0.104	245	19.4	5.26	14.7	6.36	3.07	0.48
4	0.143	245	24.6	2.51	11.3	8.73	3.57	0.41
5	0.101	no	no	1.68	8.4	6.06	3.37	0.56
6	0.038	245	14.0	3.12	10.6	3.40	1.86	0.55

Table 2. Average and mean values of inflows and outflows of the chemostat runs (1–6) indicating the repeatability of the experimental set up. Values are based on two consecutive measurements on two successive days. In the runs 1 and 2 the same medium was used. EtAc = ethyl acetate, MetxP = 1-methoxy-2-propanol, EtxP = 3-ethoxy-1-propanol

Run	Sample	EtAc (g/L)	2-Propanol (g/L)	Ethanol (g/L)	1-Propanol (g/L)	MetxP (g/L)	Etxp
1	Medium	0.63 ± 0.03	0.38 ± 0.03	6.02 ± 0.03	0.22 ± 0.04	0.19 ± 0.06	0.69 ± 0.02
1	Chemostat	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.11 ± 0.03	0.55 ± 0.02
2	Chemostat	0.03 ± 0.05	0.04 ± 0.06	0.0 ± 0.0	0.0 ± 0.0	0.14 ± 0.05	0.08 ± 0.03
3	Medium	0.58 ± 0.03	0.35 ± 0.03	5.73 ± 0.1	0.23 ± 0.05	0.15 ± 0.05	0.63 ± 0.06
3	Chemostat	0.0 ± 0.0	0.08 ± 0.02	0.0 ± 0.0	0.0 ± 0.0	0.13 ± 0.04	0.08 ± 0.03
3	Permeate	0.0 ± 0.0	0.16 ± 0.02	0.0 ± 0.0	0.0 ± 0.0	0.12 ± 0.03	0.08 ± 0.02
4	Medium	0.58 ± 0.04	0.35 ± 0.02	5.63 ± 0.01	0.21 ± 0.04	0.15 ± 0.07	0.62 ± 0.05
4	Chemostat	0.0 ± 0.0	0.3 ± 0.01	1.4 ± 0.01	0.08 ± 0.02	0.11 ± 0.02	0.59 ± 0.03
4	Permeate	0.0 ± 0.0	0.29 ± 0.02	1.35 ± 0.02	0.07 ± 0.02	0.11 ± 0.03	0.59 ± 0.02
5	Medium	0.86 ± 0.06	0.41 ± 0.02	6.91 ± 0.21	0.22 ± 0.01	0.07*	0.63 ± 0.11
5	Chemostat	0.14 ± 0.02	0.25 ± 0.01	3.4 ± 0.22	0.08 ± 0.01	0.06*	0.6 ± 0.09
6	Medium	0.84 ± 0.06	0.39 ± 0.01	6.64 ± 0.31	0.2 ± 0.01	0.06*	0.59 ± 0.13
6	Chemostat	0.12 ± 0.02	0.19 ± 0.01	1.11 ± 0.07	0.02*	0.05*	0.52 ± 0.09
6	Permeate	0.72*	0.23 ± 0.01	1.1*	0.02*	0.05*	0.08 ± 0.02

*Value missing.

eventually gave rise to strong wall growth, slime formation and unpredictable oxygen consumption. Eventually, this blocked the air stream flow into the column and increased pressure in the bioreactor unit, which resulted in increased overflow. The oxygen requirement of the mixed culture was very high and there was no stirrer device in the bioreactor unit. Thus the waste gas coming from the printing press carried out the agitation, but the reactor suffered from a weak oxygen transfer rate. Due to these problems we concentrated on improving the role of bioreactor unit in this study. Smaller volume of the bioreactor unit would improve the oxygen transfer rate and decrease the evaporation of VOCs. In addition, using a well known monoculture would increase the controllability and predictability of the VOCs removal process.

In this study we have demonstrated that a monoculture of *C. guilliermondii* can remove all the components detected from printing press exhaust gas. The yeast monocultures were highly controllable and the chemostat cultivations did not suffer from strong wall growth or blockages in the recycling unit or on the electrodes during the runs. The cell yield of 13–15 g dwt/C-mole of VOCs is comparable to cell yields with xylose as a carbon source with *C. guilliermondii* (Granström et al. 2001). The removal rate of VOC-carbon in the chemostat experiment (run 3) was 7060 C-mmol/g dwt/h (Figure 3) corresponding to 0.445

g C/L/h. The exhaust gas stream rate sampled and channelled to the bioscrubber column ranged from 1.68–3.72 m^3/h giving a maximal VOC-carbon removal rate of 12.5 g C/h. In order to remove the VOC-carbon from the sampled exhaust gas stream the working volume of the bioreactor unit could have been decreased from the original 120 L down to 28 L using the conditions of run 3. This working volume would be 76% smaller than the working volume of the bioreactor unit in the actual pilot plant.

The differences in the oxygen consumption between these two yeast strains suggest different metabolic pathways, when utilising VOCs. It is possible that *S. cerevisiae* utilises these compounds mainly through alcohol dehydrogenase enzymes (ADH) with acetate as an intermediate. Acetate is then metabolised in the TCA cycle at the expense of oxygen consumption and carbon dioxide production, whereas *C. guilliermondii* could have alternative pathways based on the lower RQ values. The most difficult compound to degrade for both yeast strains was 1-methoxy-2-propanol. It is possible that the cells suffered from oxygen limitation during recycling. This might have had an influence on the poor consumption of methoxylated- and ethoxylated-propanols. Some acetate accumulated during the runs, but no other metabolites were produced.

The volumetric removal rate could be improved by increasing the dilution rate or by cell recycling. Furthermore, through recycling it was possible to increase the volume of the bioreactor and enhance the removal of VOCs. It is conceivable that a homogenous culture might well be suited to this type of an environmental problem where pollutant concentrations are too low to be removed with any currently economical feasible method.

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