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SPECIFICITY OF ACCLIMATED BIODECOLOURISATION CULTURE TO COSUBSTRATE STRUCTURE

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SYNOPSIS

Experiments on using acclimated culture were carried out to determine if compounds other than glucose could be effective as cosubstrate in biodecolourisation of an azo dye. The role of cosubstrate was found to be not structure dependent hence cometabolism was not likely to be involved in biodecolourisation. This finding also offers opportunities for use of carbon sources other than glucose and for mixing of wastewaters to facilitate biodecolourisation.

SINOPSIS

Kajian menggunakan kultur tersesuai telah dilakukan untuk menentukan samada sebatian selain daripada glukosa boleh digunakan dalam penyahwarnaan pewarna azo. Hasil kajian menunjukkan peranan substrat kedua ini tidak tergantung kepada strukturnya dengan itu mekanisme penyahwarnaan mungkin tidak melibatkan kometabolisme. Penemuan ini juga membuka peluang bagi penggunaan sumber karbon selain daripada glukosa dan bagi pencampuran air buangan berlainan untuk mengendalikan penyahwarnaan.

INTRODUCTION

Decolourisation via chemical or physical processes constitute a large cost in treatment of dyed wastewater due to the costs of chemicals and disposal costs of the resultant sludge. Large volumes of water are used in such industries, notably in the textile finishing industries. Biodecolorisation would greatly reduce wastewater treatment costs as biological processes are cheaper due to small chemical requirement, hence small sludge volume if any.

After decolourisation the wastewater may be further treated on site by itself (Kertell & Hill, 1982; Tsang, 1982) or mixed with domestic wastewater (mainly sewage) (Davies et al, 1977) or sent to

central sewage works (Kremer et al, 1982). In industrial zones, mixing of wastewaters from two or more industries may be more economical, more so if mixing enhances treatability.

A study on condition favouring biodecolourisation of azo dyes using a culture acclimated to the dyes showed that presence of glucose greatly increased decolourisation rate (Rakmi A. Rahman and Mohd Ariffin Aton, 1987). This might be due to glucose acting as a carbon source or being involved in cometabolism or both. As a carbon source it would be used for bacterial growth. In cometabolism it would be used in positioning the enzyme(s) to enable breakdown of the dye molecule, hence its role is structure dependent. The findings reported here are from an experiment to study if cosubstrates other than glucose would also be effective for decolourisation of azo dye, hence at the same time reveal if the role of cosubstrate is structure specific.

MATERIALS AND METHODS

Cosubstrates used

Only the carbon sources more likely to be used as cosubrates in biodecolourisation were considered; these were maltose, acetate and urea.

Maltose: Maltose ($C_6H_{11}O_5$-O-$C_6H_{11}O_5$) is a disaccharide produced from starch breakdown, hance may be present in textile finishing wastewater when starch is employed as desizing agent. It may also be a by-product or present in wastewaters from other industries dealing with starch. Starch is hydrolysed by α-amylases to glucose and maltose; α-amylases is widely distributed in plants and digestive tract of animals (White et al, 1968).

Acetate: After dyeing, cloth is rinsed in a bath containing 2-3 mL/L acetic acid 60% to neutralise the alkali on the cloth. After this acidification, the cloth is rinsed with overflow, soaped and rerinsed. If this last rinse bath still shows weak alkaline reaction, pH adjustment by adding 0.2-0.3 mL/L acetic acid 60% is carried out (Hoechst, 1984).Neutralisation of the caustic soda yields sodium acetate. Hence acetate is another readily biodegradable carbon source available in textile finishing wastewaters. Acetate is also a common by-product from fermentation industries.

Urea: As previously mentioned, mixing of industrial wastewaters with sewage is frequently carried out. Sewage is a complex mixture of various body wastes, detergents, etc. One prominent component of sewage is urea, a by-product of protein metabolism.

Experimental procedure

Synthetic wastewater (SWW), with each cosubstrate and dye Remazol Red B, was made using a calculated aliquot of cosubstrate concentrate (prepared using distilled water) so as to obtain SWW with 400 mg/L carbon (Table 1). BOD bottles (300 mL) with air tight ground glass stoppers (Wheaton) each carrying a magnetic bar, and the different flasks of SWW were autoclaved at 121^{O}C for 20 minutes then cooled to room temperature (27^{O}C). The seed was a four day old liquor, grown by inoculating a BOD bottle (containing SWW without dye but with 1000 mg/l glucose) with 10 ml of mixed liquour from a sequencing batch process with dye Remazol Red B and glucose in feed.

Each reactor bottle was asceptically filled with 200mL of appropriate SWW, inoculated with 40 mL seed and stoppered. Duplicates were used for each cosubstrate. The reactors were placed on a multipoint magnetic stirrer (Variomag, Germany) sitting in a water bath equipped with a thermoregulator (Thermomix 1419, Braun Germany) set to 27^{O}C.

Samples were taken at scheduled times and immediately analysed for dye concentrations and biomass concentrations using a spectrophotometer (UV 265, Shimadzu, Japan). Measurements were carried out at 506 nm (wavelength of maximum absorbance for the dye) and at 450 nm for each sample. Calculations for two wavelength measurements, using the calibration parameters preobtained for the dye and the liquour, were carried out to obtain dye and biomass concentrations. Oxidation-reduction potential (ORP) measurements were carried out several times using an ORP probe (Cole Parmer, USA) attached to an ion meter (Ionalyser 407A, Orion, USA). The probe was rinsed in alcohol followed by sterile distilled water before each use to avoid contaminating the reactor contents.

RESULTS AND DISCUSSION

The results obtained are shown in Figures 1 and 2. The results for each cosubstrate was confirmed by the almost identical results obtained for the duplicate.

Decolourisation: Decolourisation occured with all cosubstrates except in those with urea. Hence the cosubstrate was most likely used as carbon source only; decolourisation most probably did not involve cometabolism as the role of cosubstrate was not structure specific. The lag in decolourisation was shortest with glucose, as expected since the culture had been acclimated to glucose as cosubstrate. For those with maltose and acetate, after an anitial lag of about 10 h, the rate picked up very fast and the contents decolourised at about the same time as that with glucose. Hence it can be assumed that both maltose and acetate can be as effective as

glucose when used as cosubstrate for biodecolourisation. The contents with urea showed no decolourisation at all although there was a slight growth and negative ORP was measured during the culture. A possible reason is that the growth rate was too slow to effect biodecolourisation although the prerequisite condition of negative ORP (Rakmi A. Rahman et al., 1988) was present.

Growth: Growth occured with all cosubstrates, although very slowly with urea. Hence the use of urea as carbon source for this culture most probably would not maintain the population required for steady operation. The growth rate was fastest with glucose, followed consecutively by those with maltose, acetate and urea. Fast growth was expected with glucose as the culture was acclimated to it. Glucose is also a simpler sugar than maltose (a disaccharide). Glucose is generally broken down faster than acetate. Thabaraj and Gaudy (1969) found that with glucose as substrate, acids (chiefly acetic) accumulates due to glucose being broken down faster than the carbon uptake by microorganisms.

The very slow growth for urea as cosubstrate shows that the culture could not use urea carbon as efficiently for growth or that urea carbon was converted to a form not usable by the bacteria present. A possible fate of urea under unaerated condition is conversion to inorganic carbon via $(NH_2)_2CO + 2H_2O \xrightarrow{\text{urease}} (NH_4)_2CO_3$. Since the culture had been using organic carbon, it is likely that it could not take up the inorganic carbon. However, the biodegradation of urea need to be further studied to ascertain why it led to such slow growth.

Engineering significance: Since other cosubstrates were also useable, this presents opportunities for mixing the dyed wastewater with other wastes, e.g., fermentation wastes, which would not only provide the cosubstrate carbon source required for decolourisation and maintaining a steady population level but also the nutrients and enzymes which may be lacking in the dyed wastewaters. The effectiveness of acetate also means that there is always a carbon source other than glucose present in textile finishing wastewater as acetic acid is usually used for neutralisation.

The ineffectiveness of urea means that a sizeable carbon source in sewage would not contribute to growth and decolourisation if used with this acclimated culture. However, sewage is a very complex mixture of various carbon sources; hence the effectiveness of sewage itself for decolourisation when used under the unaerated condition as in this study still remains unknown and deserves investigation.

CONCLUSION

Both maltose and acetate were found to be usable as cosubstrate for biodecolourisation of azo dye Remazol Red B. As these two have quite different structures from glucose and each other, it can be assumed that biodecolourisation does not involve cometabolism and the cosubstrate was most probably used as carbon source only. This presents opportunities for the use of other easily biodegradable carbon sources as cosubstrates or for mixing of wastes to facilitate biodecolourisation.

ACKNOWLEDGEMENT

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Table 1: Composition of Synthetic Wastewater

Salt:	mg/L
NH_4Cl	600
$MgCl_2.6H_2O$	100
$MnCl_2.4H_2O$	0.5
$FeCl_3.6H_2O$	0.5
$CaCl_2$	7.5
KH_2PO_4	454
Na_2HPO_4	944
Dye	20mg/L
Cosubstrate Carbon	400mg/L

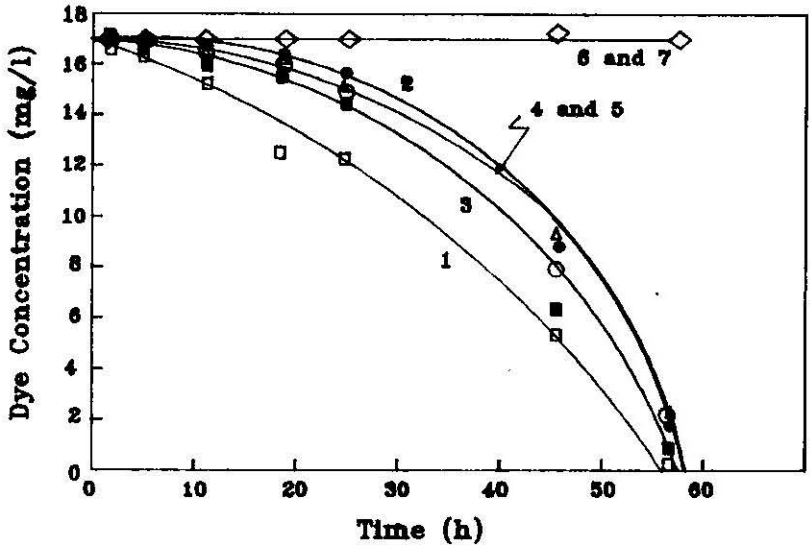

Figure 1 : Changes in Dye Concentration With Time for Substrate Glucose (1), Maltose (2 and 3), Acetate (4 and 5) and Urea (6 and 7)

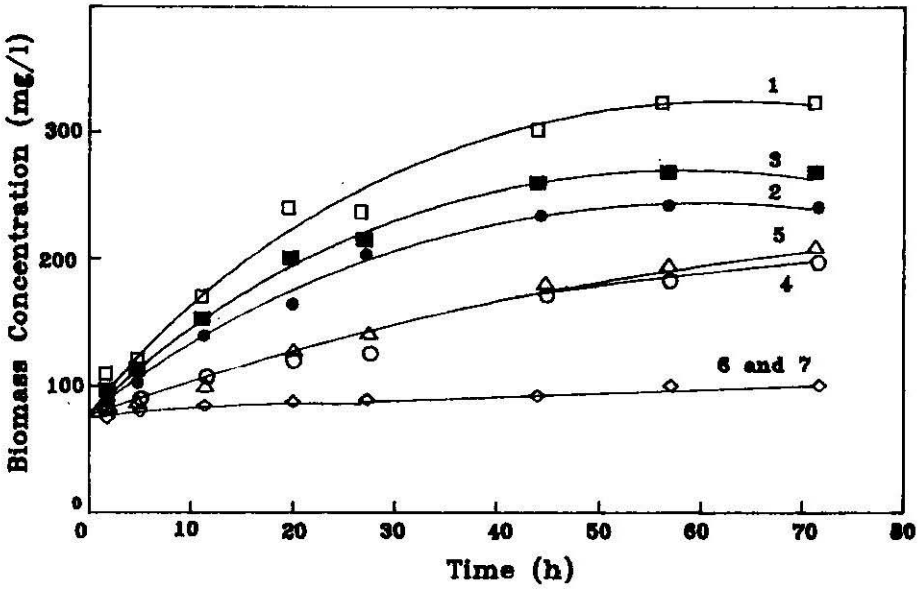

Figure 2 : Changes in Biomass Concentration With Time for Substrate Glucose (1), Maltose (2 and 3), Acetate (4 and 5) and Urea (6 and 7)