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Biodecolourisation of textile dyes by local microbial consortia isolated from dye polluted soils in ngaoundere (Cameroon)

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ABSTRACT

Azo dyes are xenobiotic and recalcitrant against biodegradation, causing environmental problems. Under certain environmental conditions, microorganisms can transform dyes to non-coloured products or completely mineralize them. In the present study, the first attempt on dye decolourisation potentials of local microbial consortia isolated from dye contaminated soils in Ngaoundere-Cameroon were determined against two azo dyes. Decolourisation rate and kinetics were monitored by spectrophotometry under different conditions. Effect of process parameters: pH, dye concentration and inoculums size on dye decolourisation rate was optimised using the full factorial design. Microbial growth and decolourisation rate were higher in shaking than static conditions. The bacteria consortium gave highest decolourisation of 91.86% for azo blue and 93.75% for azo orange within 48 hours in shaking cultures; 57.78% and 62.06% respectively after 48 hours under static condition, followed by the mixed and fungi consortium. Kinetics studies revealed the bacteria consortia had highest tendency towards decolourisation, with a greater half live value for azo blue (13.97 hours) than for azo orange (10 hours). pH out of the range 7.2-8 and dye concentrations above 100mg/l reduced decolourisation rate by the bacteria consortia while increasing inoculums size increased it. Optimum decolourisation was achieved when pH and dye concentrations were kept low while the inoculums size was high.

Keywords: Azo dye, Soil, Microbial consortia, Decolourisation, Optimisation

1 Introduction

Nowadays, in order to produce quality product, a number of dyes and auxiliary chemicals are used, producing strongly coloured effluents which has now become of critical environmental concern (Jacob and Azariah J., 2000). Majority of the colored effluents consist of dyes, released to the environment from textile dyestuff and dyeing industries. Color pollution has always being an escalating problem (Cooper, 1995), and has recently been a call for concern in Cameroon (MINEP 2009). Such pollution is particularly associated with the reactive azo dyes, because of their strong colour which leads to aesthetic problems and obstructs light penetration and oxygen transfer into water bodies (Pinheiro *et al.*, 2004), their biorecalcitrance and potential toxicity to animals and human (Hildenbrand *et al.*, 1999, Martins *et al.*, 2002), and their mutagenic activity on ground and surface waters (Sponza and Isik, 2005).
Azo dyes accounts for a significant proportion of the total dye market (Kalyani *et al.*, (2008) and comprise a diverse group of synthetic chemicals that are widely used by the textile,

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leather, food, cosmetics, and paper product industries. The general structural characteristics of azo dyes feature substituted aromatic rings that are joined by one or more azo groups (–N=N–). The annual world production of azo dyes is estimated to be around one million tons (Kalyani *et al*., 2008, Stolz, 2001), and more than 2000 structurally different azo dyes are currently in use (Vijaykumar *et al*., 2007). Due to inefficiency in the dying process, it is estimated that about 2% of these dyes are discharged in aqueous effluents during the manufacturing process while 10–50% of applied dyes are lost in textile effluents, leading to severe contamination of surface and ground waters in the vicinity of dyeing industries (O'Neill et al., 1999).

Given their beneficial technical characteristics, reactive azo dyes are used to dye more than half of the global production of cotton. Unfortunately, they are the least environmentally friendly of all dyes, generating effluents that are strongly coloured and highly concentrated in salts, thus requiring treatment before disposal. It is very difficult to treat textile industrial effluents by commonly used physical and chemical methods mainly because of their high biological oxygen demand, chemical oxygen demand, heat, colour, pH and the presence of metal ions (Kalyani *et al.*, 2008).

While much researched has been performed to develop effective treatment of dye containing wastewater, no single solution has been satisfactory for remediation of the broad spectrum of textile dye wastewater (Wallace, 2001). Several physicochemical decolourisation techniques have been reported, few of them were accepted by the textile industries (Okazakiet *et al*., 2002, Da Silva and Faria, 2003). New processes for dye degradation and wastewater treatment and reutilization are being developed, (Santos *et al*., 2007). In particular, systems based on biological processes using a large variety of bacterial strains, allow for degradation and mineralization with a low environmental impact and without the use of potentially toxic chemical substances, under mild pH and temperature conditions (Dhanve *et al*., 2008, Khalid, *et al*., 2008)**.**

Activated sludge is commonly used as an inoculum to initiate degradation, and it appears that many different microorganisms can decolorize azo dyes (Pandey *et al*., 2007) but may require a mixed community to mineralize them (Tan *et al.*, 1999, Chang *et al*., 2004). Amongst these systems, several microbial strains including *Sphingomonas spp., Pseudomonas luteola, Streptococcus faecalis, Klebsiella pneumoniae, Penicillium spp., Aspergillus spp.* have been described as being capable of reducing azo dyes (Hsueh and Chen, 2008). These microorganisms have the ability not only to decolourise dyes but also to detoxify it (Kumar *et al.*, 2006). Furthermore, it has been demonstrated that local indigenous microflora biomass is significantly better at biodegradation/biodecolourisation than commercially obtained once (Newman *et al.*, 2002), but to the best of our knowledge, no information exist about the biodecolourisation potential of local microbial isolates from Ngaoundere, Cameroon.

In view of these, he objective of this study was to evaluate the decolourisation potential of textile dyes by local microbial consortia developed from textile dye effluent contaminated soils from Ngaoundere (Cameroon). Factors affecting the decolourisation process: pH, inoculums size and dye concentration, were studied in view of optimising the decolourisation process and provide an affordable treatment technology in relation to the Cameroonian context.

2 Materials and Method

2.1 Dyestuff, chemicals and microbiological media

The textile dyes, azo blue and azo orange were provided by the Department of Applied Chemistry, ENSAI. All chemicals were of highest purity and of analytical grade. Nystatin and chloramphenicol were bought from the pharmacy. Plate count agar and Potato dextrose agar were obtained from Oxoid Company (UK).

2.2 Isolation, screening and development of dye degrading microbial consortium

The microorganisms present in soil samples from effluent disposal site of a textile dyeing house located in Ngaoundere (Cameroon) were enriched in a growth medium according to the modified method of Li *et al*., (Li et al., 2008). The enrichment medium consisted of: glucose 0.1%, yeast extract 0.05%, peptone 0.5%, NaCl 0.5%, $(NH_4)_2SO_3$ 1%, K_2HPO_4 0.02%, KH_2PO_4 0.5% and $MgSO_4.7H_2O$ 0.5%, amended with 50 mg/L of the test dyes for adaptation of the microorganisms. The medium was autoclaved at 121^oC for 30 min. After cooling, 10 g of soil samples were aseptically inoculated into three 250 ml conical flask containing 100 ml of the enrichment media. Nystatin (0.1 g/ml) was used in one flask to inhibit fungal growth for the development of the bacteria consortium, chloramphenicol (250 mg/ml) was used in the second flask to inhibit bacteria growth in the development of the fungal consortium while the third flask was used for the mixed/microbial consortium. The three flasks were incubated under shaking conditions at 180 rpm and at a temperature of 30°C. After 10 days incubation, the most effective decolourising species were screened by 48 hours incubation on Plate Count Agar and Potato Dextrose Agar amended with 50 mg/L of the test dyes for the bacteria, mixed and fungal consortium respectively. Bacteria colonies that showed a clear decolourisation zone around them were picked and reintroduced into 100 ml of freshly prepared enrichment media. Same was done for fungal species showing growth on Potato Dextrose Agar and the mixed consortium showing growth on Plate Count Agar. The three flasks containing the isolated and screened bacteria, fungal and mixed isolates were incubated at 30^oC under agitation at 180 rpm for 3 days to develop the different consortiums.

2.2.1 Microbial consortium, media and culture condition

The bacteria, fungal and mixed consortium were grown and maintained on the enrichment media proposed by Li *et al*., (Li et al., 2008) amended with 50 mg/l of the test dyes at a temperature of 30°C under agitation at 180 rpm. Decolourisation experiments were carried out in 10ml the this medium (glucose 0.1%, yeast extract 0.05%, peptone 0.5%, NaCl 0.5%, $(NH_4)_2SO_3$ 1%, K_2HPO_4 0.02%, KH_2PO_4 0.5% and $MgSO_4.7H_2O$ 0.5%) amended with 50 mg/L of the test dye. Different pH values, culture volume and dye concentration were used in the present study to investigate their effect on the decolourisation rate.

2.3 Decolourisation experiments

2.3.1 Decolourisation at static and shaking conditions

Decolourisation of individual dye by the different microbial consortium in the culture supernatants was determined using a photospectrometer (Prachi and Anushree, 2009) under shaking and static culture at optimum pH (7.2) and temperature (30 °C). 1ml inoculum from each consortium was individually introduced into separate 100 ml conical flask containing 10

ml growth medium amended with 50 mg/l of the individual dye. One set of flask was incubated under agitation at 180 rpm and temperature of 30^{o}C while the second set was incubated under stationary condition at 30^{o}C for a period of 48 hours. These served as the experimental. The control consisted flask without any microorganisms. All experiments were done in triplicates.

2.3.2 Analytical methods for dye decolourisation studies

Aliquots (3 ml) of the culture media were withdrawn at time intervals of 6 hours over 48 hours and centrifuged at 7000 rpm for 15 min. Decolourisation was quantitatively analyzed by measuring the absorbance of the supernatant using a UV–visible spectrophotometer (Spectronic® GENESYS ™ 2PC; UNI/NRE/GPA/106) at maximum wavelength, λ_{max}, of 623 nm for azo blue and 544 nm for azo orange. The decolourisation rate was calculated using the equation (Saratale et al., 2006).

$$Dye\ decolourisation\ (\%) = \frac{initial\ absorbance - final\ absorbance}{initial\ absorbance} \times 100$$

2.3.3 Decolourisation with increased dye concentration, inoculum size and different pH values

Various concentrations of dye (50, 100, 200, 300, 400 and 500mg/l), typical of those used in studies on treatment of azo-dye wastewater effluent (Zhao and Hardin, 2007) and inoculums sizes of 10% (1 ml), 20% (2 ml), 30% (3 ml) and 40% (4 ml)) were used to examine the effect of initial dye concentration and inoculums size on the decolourisation rate. Incubation was done as described above under shaking condition. The effect of different pH values (3, 5, 7.2, 8 and 10) on decolourisation rate was also investigated under shaking condition. pH was adjusted using either HCl (0.1M) or Na_2CO_3 (0.1M). The percentage decolourisation was determined over 48hrs as described above.

2.3.4 Biodecolourisation kinetics

Decolourisation kinetics occurring in the natural environment is based on the empiric approach, reflecting the rudimentary level of the microbial population and their activity in an ecosystem. When the substrate is totally available, the biodegradation will solely depend on the microbial activity, following a logarithmic growth. On the other hand, the first order kinetics link to the population density is more realistic. This approach described by equation below was used to study the decolourisation kinetics;

$$-\frac{d(C)}{d(t)} = KC^{n}$$

Where C = concentration of the substrate (dye), t = time, K = rate constant of substrate disappearance, n = an appropriate parameter.

2.3.5 Determination of nitrates concentration (NO_3^-) in treated sample

The nitrate concentration in the treated samples was determined using the sodium salicylate method (Mathieu and Pieltain, 2003). In the presence of sodium salicylate, nitrates would give paranitrosalicylate which has yellow coloration. Due to this yellow colour, colorimetric techniques can be carried out to determine the concentration of the nitrates.

2.3.6 Full factorial design for the optimisation of the decolourisation process

The full factorial design was used to evaluate the effects of the interaction between the pH, inoculums size and dye concentration on dye decolourisation rate. Decolourisation

percentage, Y, (response) which depends on these factors was represented mathematically as:

$$Y = (X_1 X_2 X_3)$$

where X_1, X_2, X_3 are pH, dye concentration and inoculums size respectively. The number of experimentation (N) for the full factorial design given by; $N = 2^k + n_o$ where k=number of factors, 3, and n_o repetition at the centre, 4. Thus, $N = 2^3 + 4 = 12$. In other to avoid the influence of the variable being dependent on the units used, the real variables were coded as represented on table 1.

Table 1: Coded variables for full factorial design

Factors	Low level (-1)	Centre (0)	High level (+1)
pH	7.2	7.6	8
Dye concentration (mg/L)	50	75	100
Inoculums size	10	25	40

3. Results and Discussion

3.1 Screening of decolourising bacteria, fungi and mixed consortia

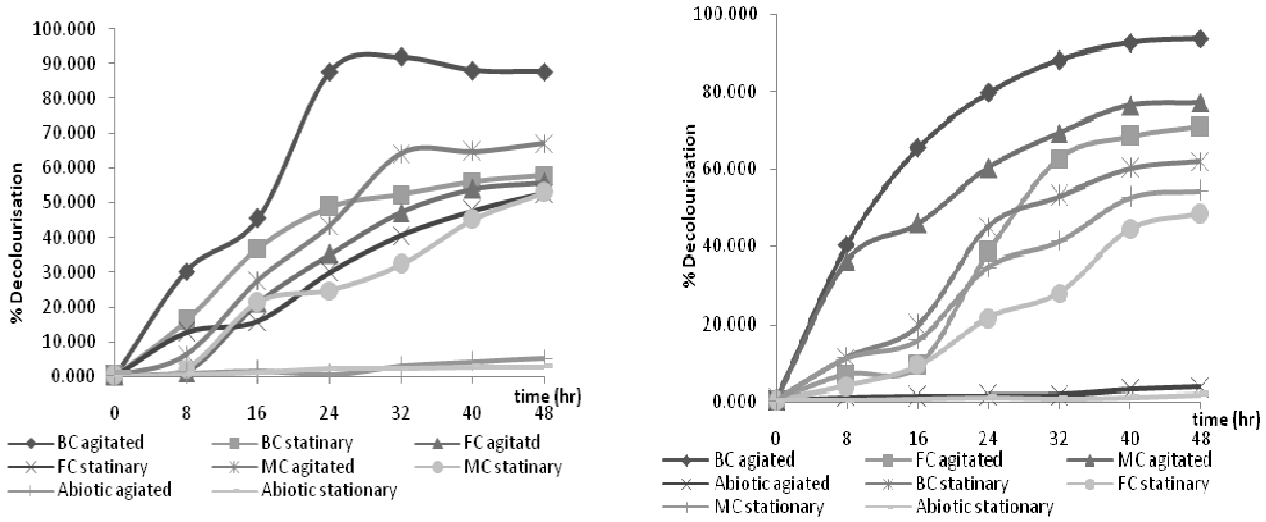
As also observed be Chen *et al.* (2003), screening on solid media after adaptation and enrichment gave effective dye decolourising isolates; bacteria colonies with decolourisation zone (Fig. 1a) and fungi showing growth and decolourisation on solid culture (Fig. 1b) which were further picked out and enriched in the liquid media to develop the deferent decolourising consortium.

a) **b)**

Figure 1: a) Bacterial colonies showing decolourisation zone on PCA amended with 50 mg/l dyes, b) Fungi showing growth on PDA amended with 50 mg/l dyes (Pictures by N. Pride Ndasi)

3.2 Decolourisation under shaking and static conditions

With higher growth in shaking condition for all the consortiums, there was a corresponding higher decolourisation rate in shaking condition than static condition for both dyes (Fig. 2).



a) **b)**

Figure 2: Decolourisation under shaking and static conditions for a) azo blue and b) azo orange

Dye decolourisation in shaking cultures varied from 55.89% to 93.75% while in static cultures, it varied from 48.41% to 62.06%. There is better oxygen transfer and nutrient distribution in shaking cultures than static cultures, therefore better microbial growth. In shaking conditions; fungi grow in spherical pellets which are uniformly suspended during agitation leading to greater contact with dye and nutrient while fungi growth in stationary cultures is in the form of mat of mycelia, which covers the whole interface of media and air, thus blocking exchanged of air (Prachi and Anushree, 2009b).

3.3 Dye decolourisation by the different consortium: bacteria, fungi and mixed consortia

The bacteria consortium exhibited highest decolourisation rate, which was higher under shaking condition, 91.86% decolourisation of azo blue after 32 hours (Fig. 3a), and 93.75% decolourisation of azo orange after 48 hours (Fig. 3c) than in static condition, 57.78% and 62.06% decolourisation after 48 hours for azo blue and azo orange respectively (Fig. 3b and Fig. 3d)

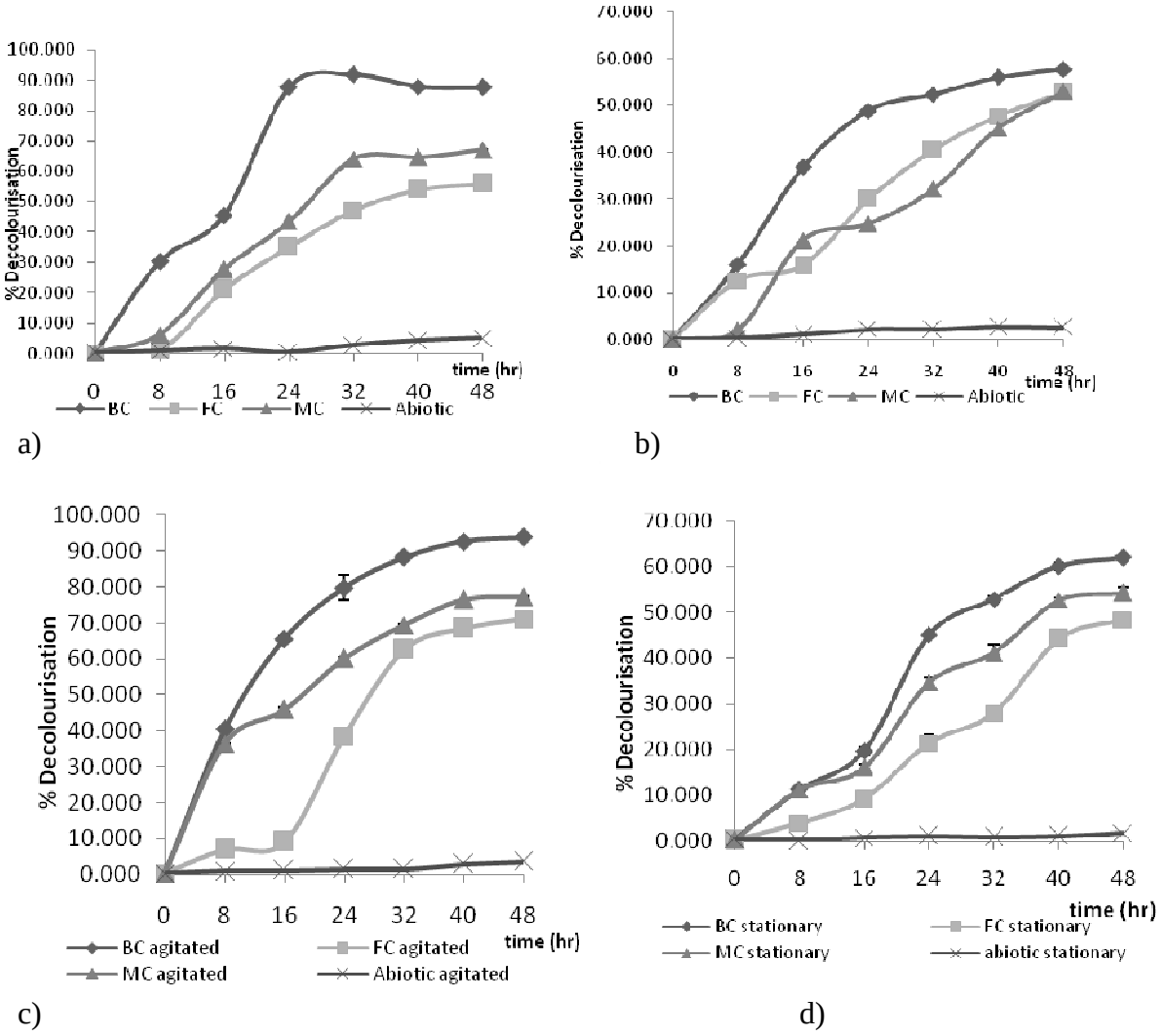


Figure 3: Percentage decolourisation of azo blue: a) Shaking condition b) Static condition and azo orange: c) Shaking condition d) Static condition

The microbial/mixed consortium on its part exhibited a maximum decolourisation of 67.04% for azo blue and 77.20% for azo orange after 48 hours under shaking conditions, with 52.94% and 54.31% decolourisation for azo blue and azo orange respectively after 48hrs under static condition. The lowest decolourisation rate was exhibited by the fungi consortium (Fig. 3) after 48 hours of incubation while the abiotic showed negligible decolourisation both in the static and the shaking conditions. Operational conditions (concentration of dye, pH and temperature) are serious drawback for biological treatment process (Fu and Viraraghavan, (2001). The ability of microorganisms to degrade azo dyes is generally correlated with the ability to synthesize enzymes, such as lignin-degrading exoenzymes, which are affected by

environmental factors such as pH, temperature and substrate concentration (Schliephake et al., 2000). While thermorphilic microorganisms have a growth temperature range of 10 to 45^{o}C, bacteria and fungi have an optimum growth temperatures between 25–30^{o}C. Fungi grow well under acidic pH values and their ligninolytic enzymes show maximal activity at low pH. The maximum degradation pH for many fungi species have been reported to be in the range of 3.5 – 4.5, while bacteria species perform well at neutral or slightly alkaline pH. Asgher *et al*., (2008) reported a fungi consortium of *Schizophyllum spp.* which decolourised 73% of solar golden yellow at a pH of 4.5 after 6 days and the efficiency decreased from 59% to 8% as pH was increased from 5 to 6 while a bacteria consortium of *Acinetobacter spp., Citrobacter freundii* and *Klebsiella oxytoca* decolourised 92% of 4-nitroaniline (and structurally different azo dyes) at a pH of 7.2 within 42 hours under aerobic conditions with shaking, which decreased as pH varied below 7 or greater than 7.2 (Azeem et al., 2009). It can thus be concluded that, efficient dye decolourisation is observed at low pH for fungi while bacteria strive well under neutral or slightly alkaline pH at temperatures between 25-35^{o}C.

Contrary to report from some authors on the synergistic interaction between bacteria and fungi species that resulted in higher decolourisation of azo dyes (Azeem et al., 2009), an antagonistic interaction in the mixed consortium, resulting to lower decolourisation rate compared to the bacteria consortium was observed. It is possible that the fungi inhibited the maximum growth of the bacteria consortium through competition for or excretion of by-products that are toxic to the bacteria.

A spectrometric scan of the supernatant after 48 hours decolourisation with the bacteria is represented on figure 4.

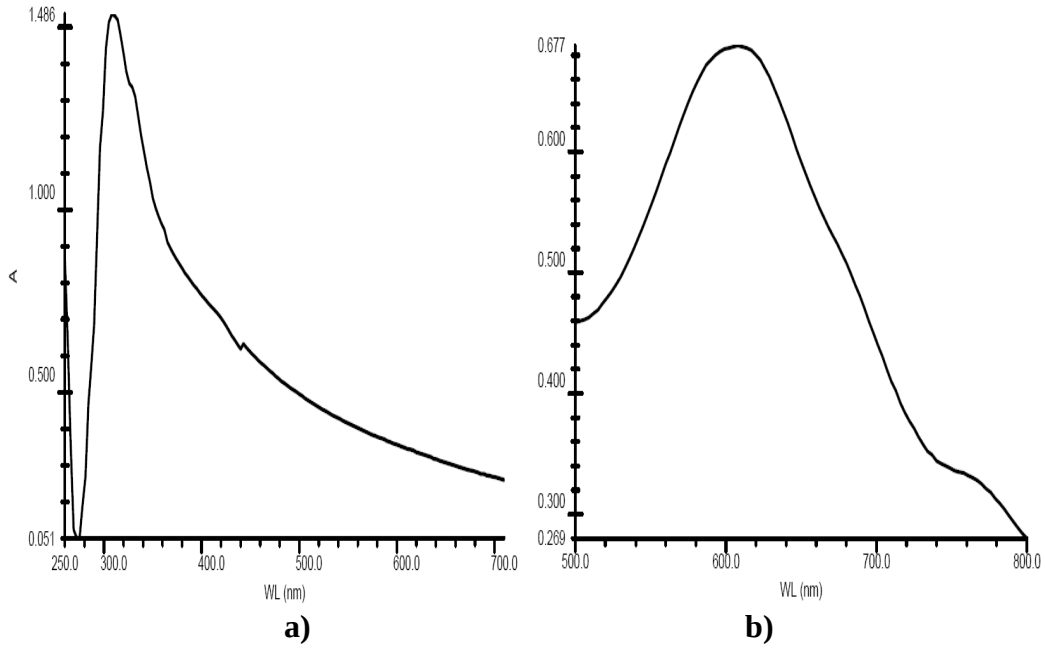


Figure 4: Spectroscopic scan of azo blue dye after 48h decolourisation; a) Bacteria consortium, b) Abiotic

3.4 Nitrate concentration (NO_3^-)

The NO_3^- concentration was determined before and after decolourisation in both dyes using the bacteria consortium under shaking conditions (Fig. 5).

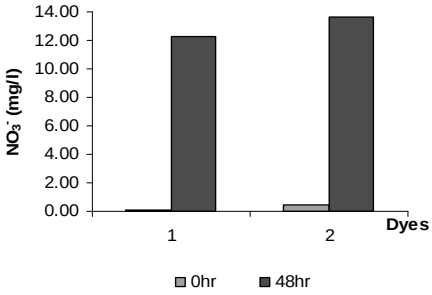


Figure 5: Nitrate concentration of dyes before decolourisation (t=0hr) and after decolourisation (t=48hrs), 1: azo blue and 2: azo orange

Nitrate concentration increased from a negligible value at the beginning, to high concentrations after 48 hours decolourisation of the dyes.

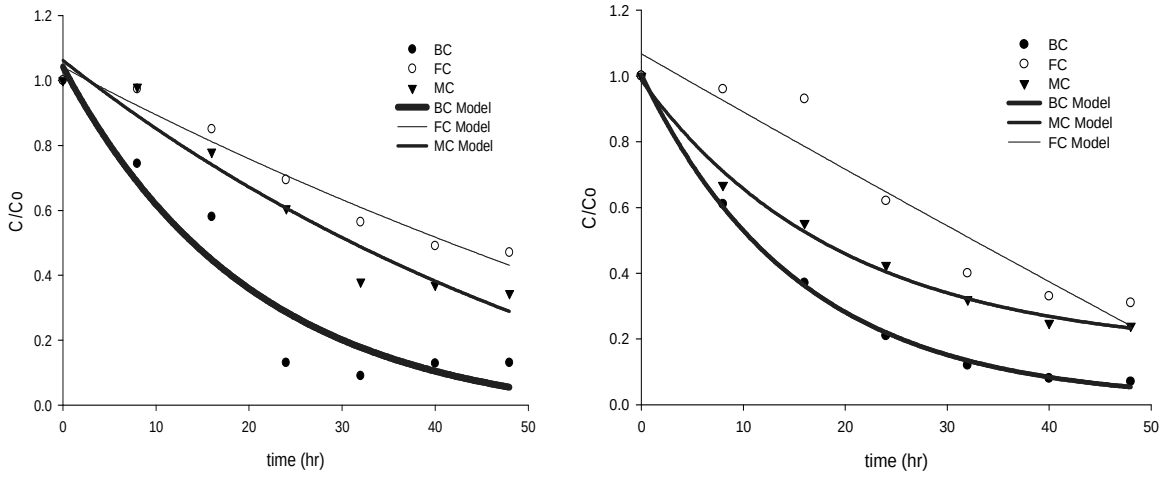
3.5 Kinetics study of the decolourisation process

The best kinetic model giving a good correlation coefficient for the dyes is the exponential model of the form;

$$\frac{C}{Co} = Y_o + Ae^{(-Bt)} \qquad \text{Eq. 2}$$

Where C and Co are dye concentration at any time, t, and at the initial time, t = 0hr, Yo, A and B are constant.
The kinetics models (Fig. 6) and kinetic parameters (Table 2) show that both dyes are degraded by all three consortia but with different correlation factors.

c



a b

Figure 6: Kinetics of azo blue (a) and azo orange (b) decolourisation by the three microbial consortia

Table 2: Kinetics parameters of azo blue and azo orange

Parameter	Bacteria consortium (BC)		Fungi consortium (FC)		Mixed consortium (MC)	
	Azo blue	Azo orange	Azo blue	Azo orange	Azo blue	Azo orange
R-Square	0.9864	0.9991	0.9657	0.9201	0.9433	0.9922
Yo	-0.0471	0.0095	-0.8880	-12.5695	-0.4530	0.1621
A	1.0894	0.9947	1.9291	13.6367	1.5150	0.8245
B	0.0493	0.0648	0.0079	0.0013	0.0149	0.0511
$T_{1/2}$ (hr)	13.97	10	31.14	32.59	41.58	17.46

The decolourisation of azo blue and orange are closely tied to the bacteria consortium as shown by the high R^2 values, with the shortest half life of 13.97 hours for azo blue and 10 hours for azo orange.

3.6 Decolourisation with increased dye concentration, inoculum size and different pH values

The bacteria consortium was unable to efficiently decolourise the increasing dye concentration from 50 mg/l to 500 mg/L with the decolourising efficiency initially increasing from 87.45% at 50 mg/l to 93.91% at 100 mg/l before dropping to 30.08% at 500 mg/l (Fig. 7a). The percentage of decolourisation increased as inoculums size was increased (figure 7 b) while the highest decolourisation rates of 84.60% and 88.70% were obtained between pH of 7.2 and 8 (fig 7c) and decreased as the pH varied away from this range.

3.7 Full factorial experimental plan to optimise azo blue dye decolourisation by the bacteria consortium

3.7.1 Determination of the model equation

The decolourisation rate from the experimental design varied from runs to runs (Table 3).
The first order mathematical model with interaction representing the decolourisation rate was given by the equation:

$$\mathbf{y = 86.554 + 3\ .223x_1 - 10.775x_2 + 13.842x_3 + 1.060x_1x_2 - 2.744x_1x_3 + 0.608x_2x_3.}$$

(R^2 = 97.36%)
The significant effects of the variable are given by the Pareto plot (Fig. 8), at a confidence level of 95%.

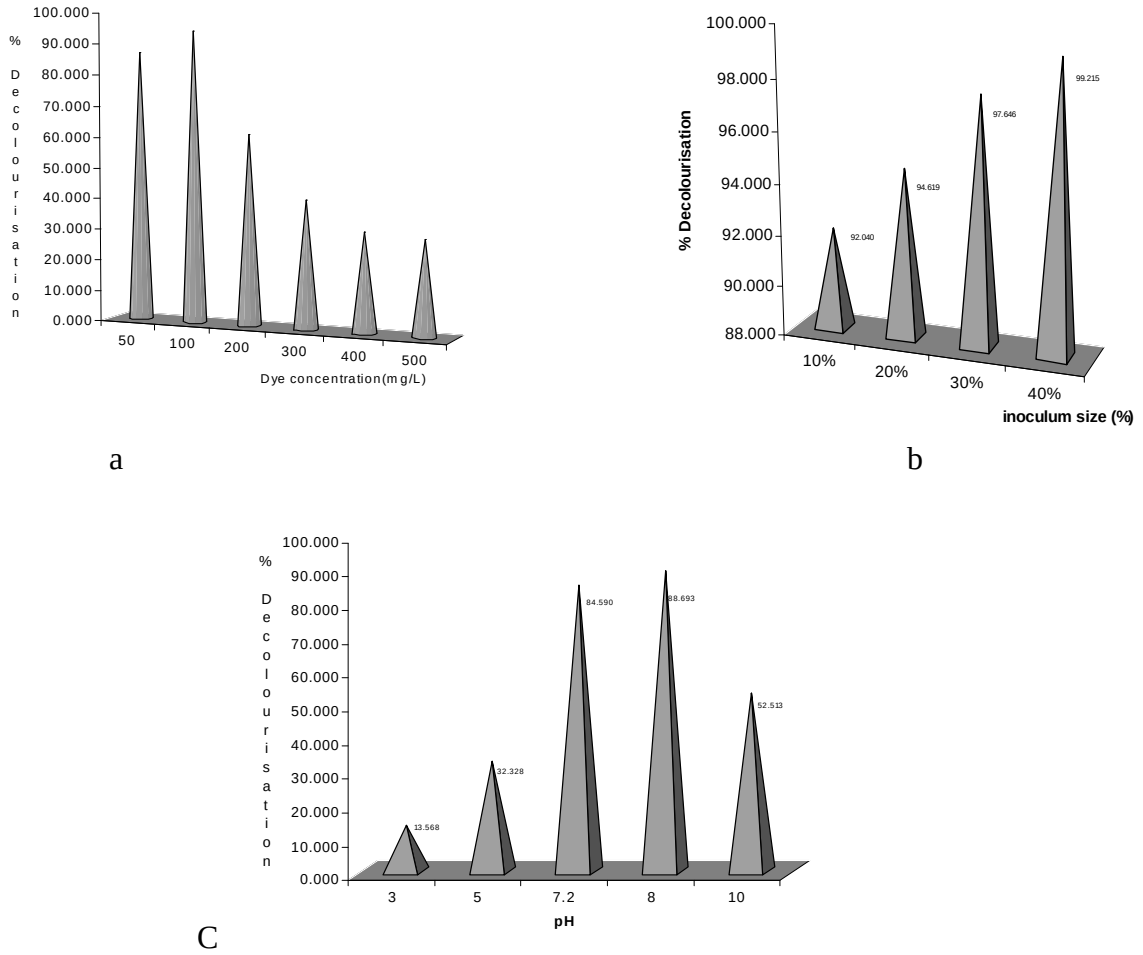


Figure 7: Effect of initial dye concentration (a), inoculums size (b) and pH (c) on decolourisation rate.

Table 3: Experimental plan and the observed responses

Experiment	Coded Variable			Real Variables			Response
	X_1	X_2	X_3	X_1	X_2	X_3	Y
1	-1	-1	-1	7.2	50	10	83.997
2	1	-1	-1	8	50	10	88.322
3	-1	1	-1	7.2	100	10	70.972
4	1	1	-1	8	100	10	78.581
5	-1	-1	1	7.2	50	40	99.394
6	1	-1	1	8	50	40	99.394
7	-1	1	1	7.2	100	40	88.747
8	1	1	1	8	100	40	89.706
9	0	0	0	7.6	75	25	85.121
10	0	0	0	7.6	75	25	84.689
11	0	0	0	7.6	75	25	85.208
12	0	0	0	7.6	75	25	84.516

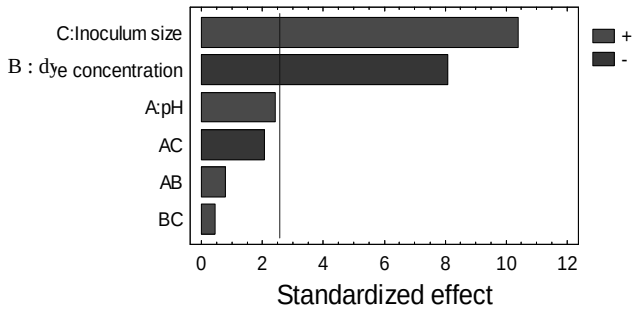
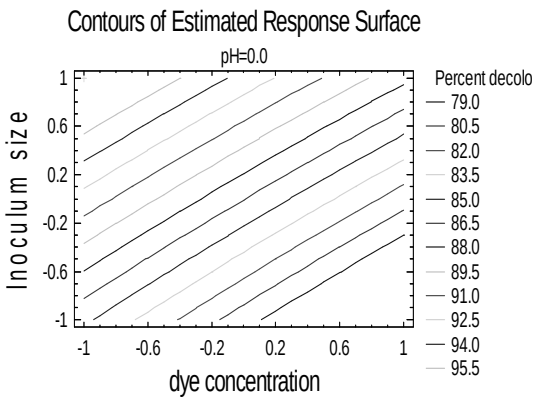


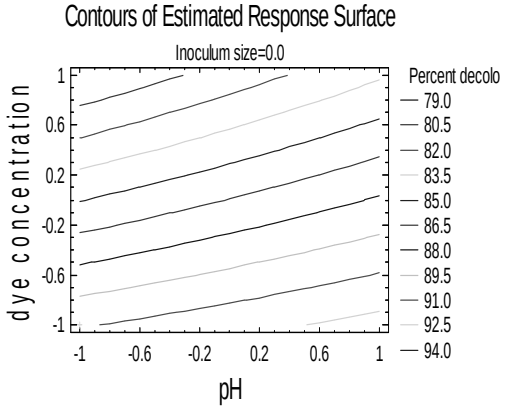
Figure 8: Pareto plot of effects of dye concentration, inoculums size and pH

3.8 Interaction of the dye concentration, inoculums size and pH

Three dimensional response of the interaction of the dye concentration, inoculums size and pH are shown on Figure 9.



a



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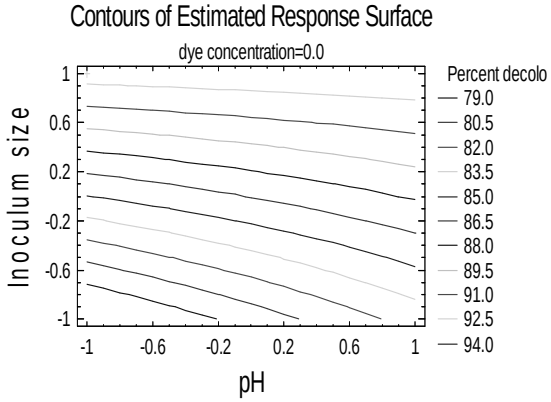

c)

Figure 9: Response surface of interaction of the dye concentration, inoculums size and pH: a) Inoculums size vs dye concentration b) Dye concentration vs pH and c) Inoculums size vs pH

3.9 Study and validation of the mathematical model

A regression plot of the theoretical and the experimental results $Y_{theoretical} = f(Y_{experimental})$ (Fig. 10) gave a regression equation of the type;

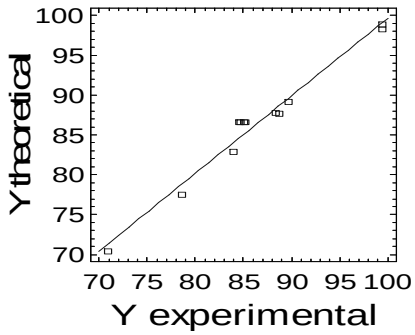

Figure 10: Regression plot of the fitted model; $Y_{theoretical} = f(Y_{experimental})$

$y = b * x + a$ where we have **y = 0.974x + 2.286.**

The high value of $R^2 = 97.358$, indicates a strong relationship between the two variables, thus the mathematical model can be validated.

4. Conclusion

The principal objective of this work was to evaluate the biodecolourisation potentials and kinetics of local microbial consortium developed from textile dye contaminated soils from Ngaoundere. Factors affecting the decolourisation process where investigated and used to optimise the decolourisation process. The three microbial consortia, bacteria, fungi and the mixed consortium all showed potentials of decolourising the tested dyes, with the decolourisation rate varying from one consortium to another. A higher decolourisation rate was observed under shaking than static condition. The bacteria consortium showed the highest decolourisation rate in both shaking (93.75 %) and static conditions (62.06%) within 48hr, followed by the mixed consortium and finally the fungi consortium. The exponential decay kinetics showed a higher tendency of decolourisation by the bacteria consortium with the shortest half life. Furthermore, the process increased with increase in inoculums size, decreased with increase in dye concentration while it was high within a pH range of 7.2 to 8. The full factorial design gave an optimal decolourisation of 98.85% when the pH and the dye concentrations were kept low while the inoculums size was high. It can be concluded that adapted local microbial isolates from textile wastewater contaminated sites can effectively be used in the aerobic treatment of these effluents before discharging into the environment.

5. References

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