Utilization of *Beta vulgaris* Agrowaste in Biodegradation of Cyanide Contaminated Wastewater

E.A. Akinpelu, O.S. Amodu, N. Mpongwana,
S.K.O. Ntwampe and T.V. Ojumu

Additional information is available at the end of the chapter

1. Introduction

Recent developments in biotechnology for agro- /agro-industrial waste utilization have identified a plethora of agricultural waste (agrowaste) that is suitable for microbial proliferation and production of a variety of high value biological products, which are useful in industrial as well as environmental applications. About 1.6 billion tons of agrowaste is reportedly generated globally per annum [25]. Considering the environmental degeneration caused by such waste, and the fact that they are readily available, research studies have been geared toward assessing the feasibility of converting such waste into value added products. Studies into the chemical and nutritional composition of agrowaste have equally identified some of them as suitable substrates for microbial cultivation [54, 40, 63, 69, 5].

In environmental bioremediation applications, microorganisms can be supported on solid agrowaste to provide the required macro- and micro-nutrients required for biofilm formation, which usually enhances the metabolic activities of the microorganisms for solubilization and biodegradation of contaminants, some of which are known to be potential human carcinogens [18, 22]. The paradigm shift from conventional substrates such as refined glucose, to unconventional substrates such as solid agrowaste or agro-industrial waste could be due to the fact that the latter mitigates operational costs, particularly for large-scale processes. Nutrients are considered the largest expense in industrial bioprocesses whereby the fermentation medium can account for a large proportion of fermentation costs [10, 39, 60]. Suitable agrowaste such as orange peel, apple pomace, wheat bran, sugar cane bagasse, wheat bran, soybean oil cake, jatropha curcas, whey waste, and *Beta vulgaris*, have been identified to support microbial growth and the synthesis of metabolites which can catalyze a number of reactions under suitable conditions[46, 42, 62, 5].

One of the most common wastewater pollutants is cyanide. It is usually released through various anthropogenic activities in the form of industrial effluent discharged from numerous industries. Another incessant anthropogenic source of cyanide deposition into the environment is through petroleum oil processing and its derivatives. Naturally, hydrocarbon oils such as petroleum contain cyano group compounds, which react with metals during thermal cracking operations to form metal cyanide complexes that culminate in wastewater [14]. Many of these cyanide complexes are known to be highly unstable, mainly due to thermal instability, thus releasing free cyanide into the environment under high temperature. It has been reported by Acheampong *et al.* [1] that, cyanide concentrations from facilities that serve industrialized areas could have cyanide concentration higher than 21.6 mg F-CN/L. Cyanide exposure is known to result in neurological disorders and thyroid abnormalities in humans [69, 55]; hence, a robust and economically feasible bioremediation process using renewable resources (agrowaste), i.e. an environmentally benign approach, is necessary to ensure a sustainable and an effective bioremediation process for cyanide deposited into the environment.

It is common to use oxidation methods for cyanide degradation and its complexes, such as the use of metal catalyzed hydrogen peroxide, and alkaline chlorination processes, including removal by ion-exchange resin [17]. This approach, though effective, has some drawbacks that are of major concern. The excess reagents used in the treatment tend to further pollute the environment, as well as increase operational costs. In addition, due to municipal regulations in some countries, the application of chemical methods on a large scale is not permissible. Considering that cyanide in wastewater is undesirable, if present, it must not exceed the discharge limit of 0.01 mg F-CN/L [23]. Thus, cyanide degradation using biotechnological processes is desirable.

It has been shown that several microorganisms such as algae, bacteria, and fungi, can produce enzymes that are capable of degrading free cyanide, cyanide complexes and by-products produced [3, 24, 33, 59]. Recently, studies have established sustainable cyanide biodegradation processes using various microorganisms such as *Klebsiella sp.*, *Pseudomonas sp.*, *Acinetobacter sp.*, *Bacillus sp.*, and many others [41, 58]. A fungal specie - *Fusarium oxysporum*, has equally been reported for its ability to produce enzymes such as nitrilase which readily hydrolyses cyano-compounds into a corresponding weak acid and ammonium-nitrogen, thus bioremediating the contaminated wastewater, with both the acid and ammonium-nitrogen produced being consumed for metabolic functions [37, 32]. Several agrowaste have also shown to be effective substrates for the cultivation of microorganisms and for the biodegradation of cyanic compounds [15, 30, 49].

The application of agrowaste as a substrate in cyanide biodegradation systems is particularly promising, as reported by Santos *et al.* [62]. Having a readily accessible waste material, microorganisms will be able to produce enzymes suitable for bioremediating contaminants in wastewater [59]. Besides their application as effective biosorbents, agrowaste can serve as a sole substrate for bioremediation purposes, on condition that it is compatible to the microbial community to be used [67, 45, 17, 16]. In South Africa, approximately 10 million tons of agrowaste is generated per annum [53], of which 96% is classified as pre-consumer waste

(Figure 1). This is a large quantity of waste for a developing economy and should be put into profitable use to safe our environment.
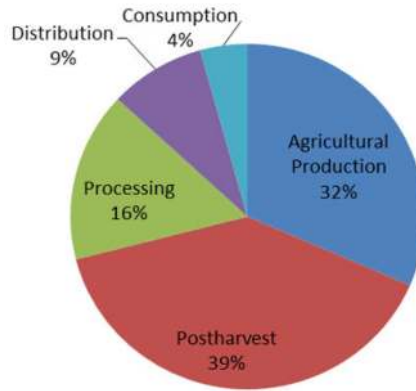

Figure 1. Classification of agrowaste production in South Africa [53]

2. Application of *Fusarium sp.* and *Beta vulgaris* in cyanide biodegradation

Fusarium sp. are widely distributed in environmental samples, particularly in soil. They can cause spoilage of agricultural produce and produce mycotoxins which contaminate cereal crops, affecting human and animal health, if the mycotoxins enter the food chain. *Fusarium sp.* has also been found useful in the hydrolysis of starch. The hydrolysed agricultural produce can be used to sustain the production of extracellular enzymes such as pectinase, cellulase, xylanase, amylase, and organic acids [43]. The fungus is also known for the production of cyanide hydratase and nitrilase including cyanidase. *Fusarium sp.* has been identified as having the ability to degrade cyanides through hydrolysis at varying temperature and pH, then metabolise the by-products as either nitrogen and carbon sources, respectively [52, 31]. The cyanide hydratase, converts the cyanide to amide products and ammonium-nitrogen while the nitrilase hydrolyse cyanide to produce a carboxylic acid [50]. Compared with other enzymes derived from bacteria, nitrilase and cyanide hydratase are of higher activity and can degrade various cyanides [59].

Beta vulgaris waste consists of water, carbohydrates, minerals and proteins which makes it a suitable substrate for microbial growth in the production of high value compounds [5, 45]. However, limited studies have shown its potential as a feed stock and solid support in a bioreactor for the biodegradation of cyanide in the presence of heavy metals [46]. Additionally, hydroxyl functional groups found in *B. vulgaris* waste can act as pseudo-catalysts for the conversion of cyanide to ammonium-nitrogen. Alhough the free hydroxyl functional group is a weak acid, they are able to deprotonate to produce alkoxides in the presence of a strong base like cyanides especially at high alkaline pH (Figure 2) [31, 62].
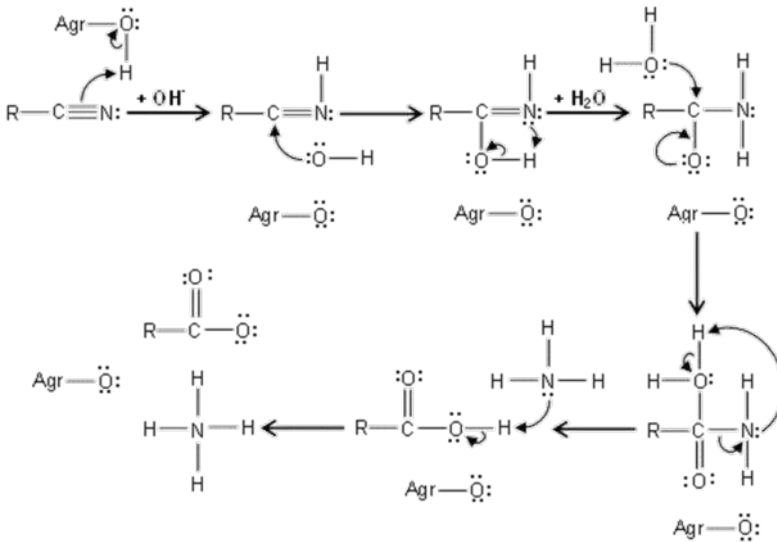
Figure 2. Pseudo-catalyst conversion of cyanide by free hydroxyl functional groups [61]

3. Biodegradation of cyanide by *Fusarium oxysporum* grown on *Beta vulgaris*

A number of different studies report on the application of cyanide degrading fungi. For instance, white rot fungi, *Trametes versicolor*, have been shown by Cabuk *et al.* [9] to tolerate cyanide concentration up to 130 mg F-CN/L, with complete degradation observed within 42 hours to produce minute quantities of ammonium-nitrogen (5.24 mg NH_4^+-N/L). Fourteen cyanide degrading fungi were examined by Pereira *et al.* [57] such as *Fusarium sp.* including *Aspergillus sp.* by Santos *et al.* [57, 62], and were found to tolerate cyanide concentration up to 520 mg F-CN/L. A list of other cyanide degrading species including degradation conditions are shown in Table 1.

There has been limited emphasis on the effect of carbon or nitrogen sources used in the biodegradation of cyanide. The viability of the agrowaste depends on the type of bioremediation required and the microorganism used. When the cultivating conditions are conducive, the minerals, proteins, carbohydrates and water in the agrowaste become easily accessible to the microorganisms [46]. Monosaccharides such as mannose, glucose and fructose present in the agrowaste can effectively support and/or enhance microbial growth [2]. Other overriding factors which directly influence cyanide degradation include exposure to direct sunlight, temperature and pH. Cyanide compounds are soluble in water, thus dissociate and evaporate easily at low pH (i.e. pH<9) while under high salinity, the solubility decreases. Also at neutral

pH, weak-acid dissociable (WAD) cyanides such as copper or zinc cyanide complexes, if present in a high concentration, dissociate, releasing a cyano group. Similarly, the reduction in temperature reduces the activity of microorganisms used in bioremediation. A number of studies have proven that, at low temperature (below 10ºC), growth of microorganisms is inhibited, resulting in low removal rates of contaminants such as ammonium-nitrogen, nitrates and cyanide [72, 29, 73].

In this study, the biodegradation of cyanide in the presence of heavy metals (arsenic, copper, lead, iron and zinc), using *Fusarium oxysporum* grown on *B. vulgaris* waste as the sole carbon source, without any buffer solution, was investigated. The effect of temperature and pH on cyanide degradation with minimal ammonium-nitrogen production was studied using a response surface methodology.

4. Materials and methods

The experiments were carried out in batch cultures. *B. vulgaris* waste was milled to ≤ 100 μm. A broth of 0.5 g of milled waste in 10 mL distilled water was autoclaved at 116°C for 15 min to prevent thermal breakdown of reducing sugars [51]. To the waste broth, wastewater (20 mL) with 1 mL of a spore solution (2.25×10^6 spore/mL) of *Fusarium oxysporum* was added to the *B. vulgaris* broth. The wastewater used had characteristics similar to the goldmine wastewater reported by Acheampong *et al.* [1] having metals such as arsenic, iron, copper, lead and zinc. The mixture was incubated for 48 hours in a rotary shaker at 70 rpm at the desired temperature and pH (- see Table 2). After this, KCN in distilled water, was added to make a final cyanide concentration of 500 mg CN^-/L in the mixture. Thereafter, the mixture was incubated for a further 72 hours at 70 rpm at the desired temperature (- see Table 2). All experiments were carried out in duplicate in airtight multiport round bottom Erlenmeyer flasks (n = 28; final volume of 51 mL). Cyanide (CN) (09701) and ammonium-nitrogen (NH_4^+-N) (00683) test kits (MERCK®) were used to quantify the residual free cyanide and ammonium-nitrogen concentrations using a NOVA 60 spectroquant. Free cyanide volatilised was accounted for using the mass balance equations below:

$$CN^-_s - \left(CN^-_r + CN^-_v\right) = CN^-_b \tag{1}$$

$$CN^-_v = CN^-_{vo} - CN^-_{vf} \tag{2}$$

where CN^-_s is the initial free cyanide concentration in the culture broth; CN^-_r is the measured residual free cyanide after incubation; CN^-_v is the volatilised free cyanide during incubation; CN^-_b is the bioremediated free cyanide; CN^-_{vo} is the initial free cyanide in control cultures (500 mg F-CN/L); and CN^-_{vf} is the final free cyanide in control cultures. The control was prepared under the same conditions as other cultures without the *Fusarium oxysporum*.

Microorganism	C-source	N-source	Temperature (°C)	pH	Reference
Fusarium oxysporum	*Beta vulgaris*	**KCN**	**30**	**11**	**This study**
Agrobacterium tumefaciens	Starch	KCN	-	7.2	[58]
Aspergillus awamori	*Citrus sinesis* extract	KCN	40	8.84	[62]
Baccillus pumilus	Glucose	KCN	40	8.5-9	[64]
Baccillus stearothermphilus	-	NaCN	27±2	7.8	[6]
Burkholderia cepacia	Fructose, glucose, mannose	KCN	30	10	[2]
Citrobacter sp., Pseudomonas sp.	Sugarcane molasses, glucose	$[Cu(CN)_4]^{2-}$, $[Zn(CN)_4]^{2-}$	35	7.5	[56]
Cryptococcus humicolus MCN2	Glucose	KCN	25	7.5	[36]
Eschericia coli	Glucose	KCN	30	9.2	[26]
Fusarium solani	Glucose	$K_2Ni(CN)_4$, KCN	25	7.0	[8]
Fusarium solani	Yeast	KCN	30	9.2-10.7	[19]
Fusarium oxysporum	Glucose	KCN	25	8.0	[57]
Fusarium oxysporum immobilised on sodium alginate	Formamide	Cyanides	25-30	8	[11]
Gloeocercospora sorghi, Stemphylium loti	Glucose	KCN	35, 28	5.3-5.7, 7.0	[48]
Klebsiella oxytoca	Glucose	KCN	30	7	[34]
Klebsiella oxytoca immobilised cell	Alginate and cellulose triacetate	KCN	30	7	[13]
Mixed culture of bacteria	Glucose	CN⁻ $_{WAD}$	22	7.0	[70]
Mixed culture of bacteria immobilised on ultrafiltration membranes	Phenol	Cyanides	25	-	[35]
Pseudomonas fluorescens	Glucose	Ferrocyanide	25	5	[21]
Pseudomonas fluorescens immobilised on calcium alginate	Glucose	Ferrocyanide	25-35	4-7	[20]
Pseudomonas fluorescens immobilised on zeolite	Zeolite	Tetra-cyano-nickelate (II)	30	-	[66]
Pseudomonas pseudoalcaligenes CECT5344	CH_3COONa	NaCN	30	9.5	[38]
Pseudomonas putida BCN3	Glucose	$[K_2[Ni(CN)_4]]$	30	-	[63]
Pseudomonas putida immobilised on sodium alginate	NaCN	NaCN	25	6.7	[7]
Pseudomonas putida immobilised on sodium alginate	NaCN, sodium alginate	NaCN, Cyanates and thiocyanates	25	7.5	[12]
Pseudomonas stutzeri AK61	-	KCN	30	7.6	[68]
Pseudomonas sp.(CM5, CMN2)	Glycerol	CN⁻ $_{WAD}$	30	9.2-11.4	[4]
Stemphilium loti		KCN	25	6.5, 7.5	[27]
Trametes versicolor	Citrate	KCN	30	10.5	[9]
Trichoderma sp.	Glucose	CN⁻	25	6.5	[24]
Scenedesmus obliquus	NaCN	NaCN	-	10.3	[33]
Rhodococcus UKMP-5M	Glucose	KCN	30	6.6	[41]

Table 1. Cyanide degrading microbial species using different nutritional sources under different temperature and pH conditions

Run	Temperature (°C)	pH
1	19.5	8.5
2	9	11
3	19.5	8.5
4	30	11
5	30	6
6	19.5	8.5
7	9	6
8	19.5	8.5
9	19.5	12.04
10	34.35	8.5
11	4.65	8.5
12	19.5	4.96
13	19.5	8.5
14	19.5	8.5

Table 2. Experimental variation of pH and temperature

The response surface methodology was used for the statistical design of the experiments to assess the influence of temperature and pH for optimal degradation of cyanide. A central composite design was used for the determination of optimal operating conditions with a minimum residual ammonium-nitrogen as one of the objectives. Design Expert software® version 6.0.8 (Stat-Ease Inc., USA) was used to generate the experimental runs.

Run	A	B	F-CN degraded (mg F-CN/L)		Residual NH_4^+ (mg NH_4^+-N/L)	
			Experimental value	Predicted value	Experimental value	Predicted value
1	0	0	239	238.86	210	219.14
2	-1	1	229	196.59	100	183.50
3	0	0	239	238.86	210	219.14
4	1	1	250	250.29	40	83.43
5	1	-1	135	167.62	320	222.79
6	0	0	239	238.86	210	219.14
7	-1	-1	127	126.92	128	70.86
8	0	0	239	239.14	210	200.86
9	0	1.414	263	285.75	210	117.41
10	1.414	0	196	172.77	100	135.11
11	-1.414	0	83	106.02	120	98.52
12	0	-1.414	201	178.03	30	136.30
13	0	0	239	239.14	210	200.86
14	0	0	239	239.14	210	200.86

A and B represent coded level of variables.

Table 3. Coded experimental design variables and the corresponding response

The results (Table 3) indicated a variation in responses measured. There was appreciable degradation of cyanide in Runs 9, 4, 1, 3, 6, 8, 13, and 14, with the highest cyanide degraded being 263 mg F-CN/L (Run 9) and the lowest (83 mg F-CN/L) being observed for Run 11. However, both cases had a high residual ammonium-nitrogen of 210 mg NH_4^+-N/L and 120 mg NH_4^+-N/L, respectively. Both Runs 9 and 11 were axial points. Run 9 with an extremely high pH resulted in high residual ammonium-nitrogen while Run 11 with an extremely low temperature was observed to have minimal microbial activity despite the presence of a suitable quantity of *B. vulgaris* used as a carbon source. A similar scenario had earlier been reported by Zilouei *et al.* [72] and Zou *et al.* [73], whereby a low temperature was found to inhibit the growth of microorganisms, thus resulting in low removal of contaminants (ammonium-nitrogen, nitrate and nitrite). On the other hand, Runs 1, 3, 4, 6, 7, 8, 13 and 14 had up to 99% correlation with the predicted values for cyanide degradation which indicated a high accuracy of the model (Equation 4) used for predicting cyanide degradation. However, only Runs 4 and 7, which showed minimal residual ammonium-nitrogen presence, can be used for optimisation for a pilot scale process.

5. Statistical model analysis

The statistical model summary clarifies the fitness of the mean and quadratic models for the two responses based on the Sequential Model Sum of Squares and Lack of Fit Test. The responses were analysed using ANOVA to assess the significance of the variables in the model. A quadratic model was found to give the best fit for the experimental results.

Factor	Coeff. Estimate	DF	Standard Error	95% CL Low	95% CL High	F Value	Prob > F	Significance
Intercept	239	1	10.03	215.27	262.73	11.41	0.0029	S
A	23.6	1	8.69	3.05	44.50	7.37	0.0300	S
B	38.09	1	8.69	17.54	58.63	19.21	0.0032	S
A²	-49.87	1	9.05	-71.26	-28.49	30.40	0.0009	S
B²	-3.62	1	9.05	-25.01	17.76	0.16	0.7005	NS
AB	3.25	1	12.29	-25.81	32.31	0.07	0.7991	NS

S = significant; NS = Not significant; CL = Confidence Level; DF = Degree of freedom; "Prob > F" less than 0,05 indicates the model term is significant while values greater than 0.1 indicates the model term is not significant; Std. Dev. = 24.58; R^2 = 0.8907; Adj. R^2 = 0.8127; Pred. R^2 = -0.1858; Adeq. Precision = 10.341

Table 4. ANOVA for F-CN Reponse Surface Quadratic Model

The predicted response (Y) for the biodegradation of free cyanide in terms of the coded values was:

$$Y = 239 + 23.6A + 38.09B - 49.87A^2 - 3.62B^2 + 3.25AB \qquad (3)$$

where *A* and *B* are the coded values of temperature and pH, respectively. When coefficients with significant effects were considered, Eq. (3) became;

$$Y = 239 + 23.6A + 38.09B - 49.87A^2 \qquad (4)$$

A model reduction was appropriate since there were many insignificant model terms. Excluding these terms improved the model. The Model F-value of 11.41 for the cyanide biodegradation was significant; therefore, there was only a 0.29% chance that a "Model F-Value" this large could occur due to noise for the quadratic model. Statistically, an adequate ratio greater than 4 is desireable for measuring a signal to noise ratio; therefore, the adequate precision of 10.341 observed in this study indicates a passable signal that can be used to further navigate the design space. Figure 3 further justifies the fitness of the model with normality in the error term.

6. Representation of the response surface model

The interaction between independent variables can be studied by plotting three dimensional (3-D) curves of the response against the variables. It allows for the interpretation of experimental results and determination of optimal conditions. Elliptical contour shows the interaction between the independent variables is perfect while a circular contour indicates the variables are non-interactive [44, 47].
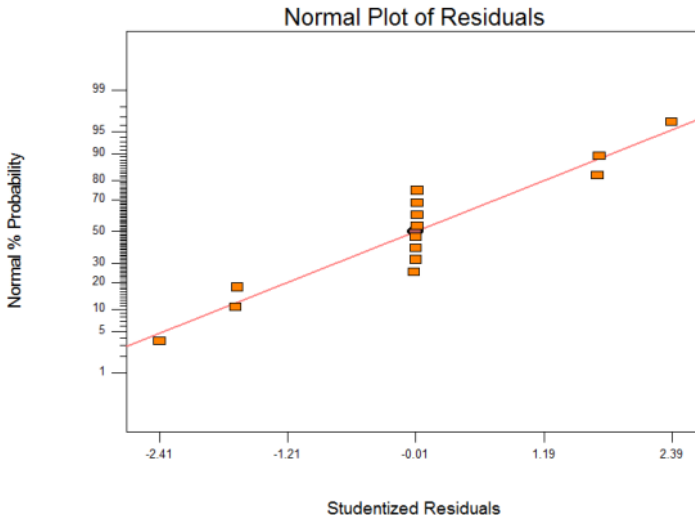

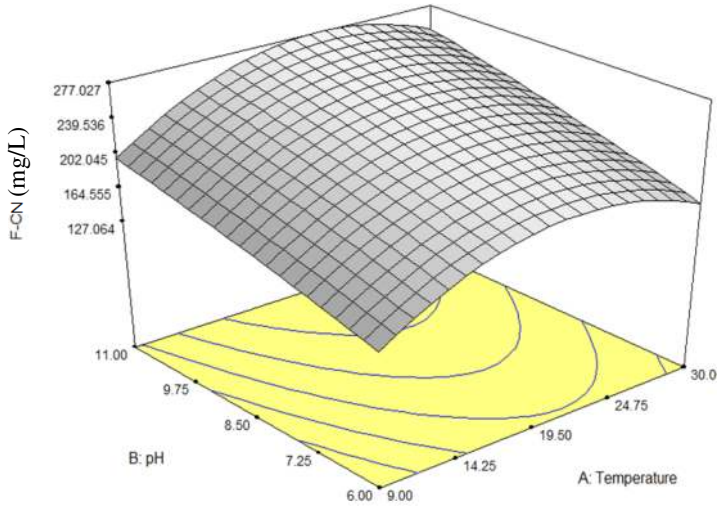**Figure 3.** Normal probability plot of the residual F-CN

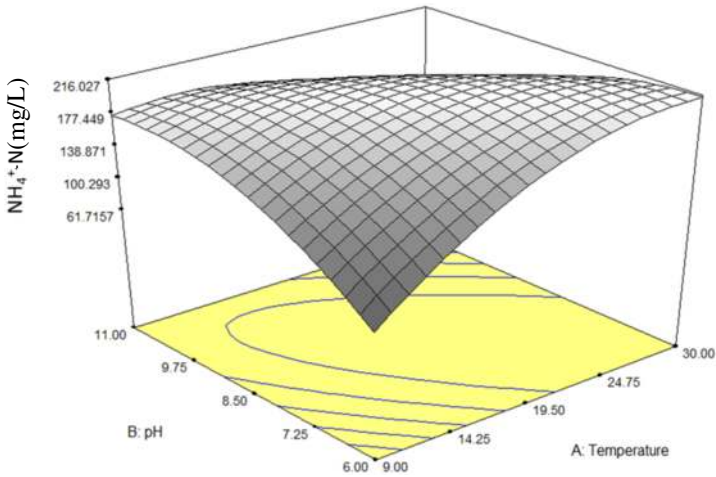**Figure 4.** D plot showing interaction of independent variables on cyanide degradation

Figure 5. D plot showing interaction of independent variables on ammonium-nitrogen formation

7. Cyanide biodegradation optimisation

The optimisation was done using the Design-Expert software® numerical optimisation option where input factors were selected to achieve a desired perfomance. The numerical optimisation

can maximise, minimise or achieve a targeted value: a single response; a single response subjected to upper and/or lower boundaries on other responses; and combinations of two or more responses. The desired goal for each variable and response is selected and the weight is chosen to show the degree of importance of individual goals. In this analysis, temperature and pH were set within range, cyanide degradation response was set at maximum while ammonium-nitrogen formation response was set at a minimum. The software gave three different solutions for this criteria with different desirability. The optimum point with the highest desirability was selected as shown in Fig.6 and 7. The optimal point with the maximum cyanide degradation of 250.436 mg F-CN/L and minimum ammonium-nitrogen formation of 74.285 mg NH_4^+-N /L was found to be at temperature of 30°C and pH of 11.
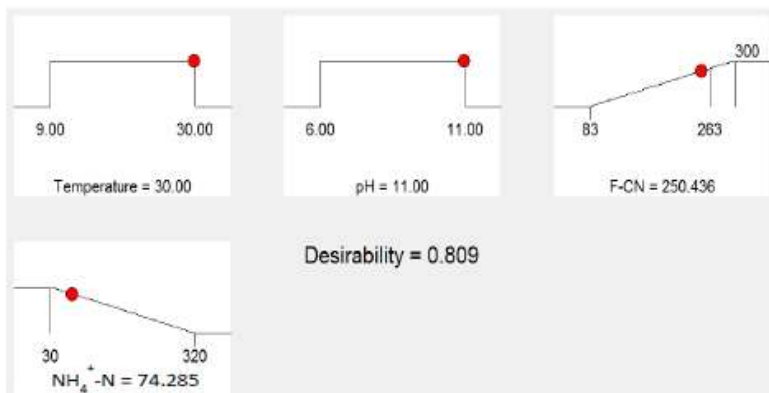
Figure 6. Desirability ramp for the numerical optimisation of cyanide degradation and ammonium-nitrogen formation
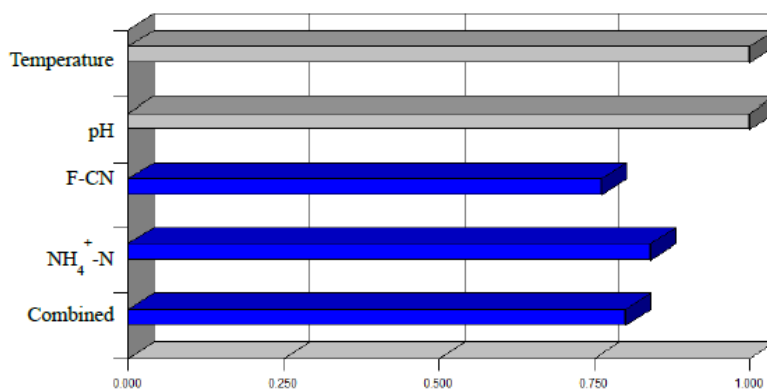
Figure 7. Desirability histogram for numerical optimisation of cyanide degradation and ammonium-nitrogen formation

8. Conclusion

Fusarium oxysporum cultures were grown on *B. vulgaris* waste to facilitate the biodegradation of cyanide, with the initial concentration of the cyanide being 500 mg CN$^-$/L. The wastewater used was similar to the effluent discharged into ponds by goldmines having metals such as arsenic, copper, lead, iron and zinc.

The response surface plot identified temperature as a more significant factor affecting both the cyanide degradation and ammonium-nitrogen formation. The ammonium-nitrogen produced can be used as a nitrogen source by the fungus.

The optimum condition for maximum cyanide degradation and minimum ammonium-nitrogen formation was found at temperature 30°C and pH of 11 where cyanide of 250.436 mg F-CN/L was degraded and ammonium-nitrogen of 74.285 mg NH$_4^+$-N/L was formed.

Acknowledgements

Author details

E.A. Akinpelu[1,2], O.S. Amodu[1,2], N. Mpongwana[2], S.K.O. Ntwampe[2*] and T.V. Ojumu[1]

*Address all correspondence to: NtwampeS@cput.ac.za

1 Faculty of Engineering, Department of Chemical Engineering, Cape Peninsula University of Technology, Cape Town, South Africa

2 Bioresource Engineering Research Group, Cape Peninsula University of Technology, Cape Town, South Africa

References

[1] Acheampong M, Paksirajan K, Lens PL. Assessment of the effluent quality from a gold mining industry in Ghana. Environmental Science and Pollution Research 2013; 20(6) 3799-3811.

[2] Adjei MD, Ohta Y. Factors affecting the biodegradation of cyanide by Burkholderia cepacia strain C-3. Journal of Bioscience and Bioengineering 2000; 89(3) 274-277.

[3]   Akcil A. Destruction of cyanide in gold mill effluents: biological versus chemical treatments. Biotechnology Advances 2003; 21(6) 501-511.

[4]   Akcil A, Karahan AG, Ciftci H, Sagdic O. Biological treatment of cyanide by natural isolated bacteria (*Pseudomonas sp*.). Minerals Engineering 2003; 16(7) 643 - 649.

[5]   Amodu OS, Ntwampe SK, Ojumu TV. Emulsification of Hydrocarbons by Biosurfactant: Exclusive Use of Agrowaste. BioResources 2014; 9(2) 3508-3525.

[6]   Atkinson A. Bacterial cyanide detoxification. Biotechnology and Bioengineering 1975; 17(3) 457-460.

[7]   Babu GRV, Wolfram J, Chapatwala K. Conversion of sodium cyanide to carbon dioxide and ammonium-nitrogen by immobilized cells of *Pseudomonas putida*. Journal of Industrial Microbiology 1992; 9(3-4) 235-238.

[8]   Barclay M, Day JC, Thompson IP, Knowles CJ, Bailey MJ. Substrate-regulated cyanide hydratase (chy) gene expression in *Fusarium solani*: the potential of a transcription-based assay for monitoring the biotransformation of cyanide complexes. Environmental Microbiology 2002; 4(3) 183-189.

[9]   Cabuk A, Unal A, Kolankaya N. Biodegradation of cyanide by a white rot fungus, *Trametes versicolor*. Biotechnology Letters 2006; 28(16) 1313-1317.

[10] Cameotra S, Makkar R. Synthesis of biosurfactants in extreme conditions. Applied Microbiology and Biotechnology 1998; 50(5) 520-529.

[11] Campos MG, Pereira P, Roseiro JC. Packed-bed reactor for the integrated biodegradation of cyanide and formamide by immobilised Fusarium oxysporum CCMI 876 and Methylobacterium sp. RXM CCMI 908. Enzyme and Microbial Technology 2006; 38(6) 848-854.

[12] Chapatwala KD, Babu GRV, Vijaya OK, Kumar KP, Wolfram JH. Biodegradation of cyanides, cyanates and thiocyanates to ammonium-nitrogen and carbon dioxide by immobilized cells of *Pseudomonas putida*. Journal of Industrial Microbiology and Biotechnology 1998; 20(1) 28-33.

[13] Chen CY, Kao CM, Chen SC. Application of Klebsiella oxytoca immobilized cells on the treatment of cyanide wastewater. Chemosphere 2008; 71(1) 133-139.

[14] Coty VF, Doll BE, Lifland PP (1980). Cyanide removal from wastewaters, Google Patents.

[15] Cramer CS. Breeding and genetics of Fusarium basal rot resistance in onion. Euphytica 2000; 115(3) 159-166.

[16] Das N, Chandran P. Microbial degradation of petroleum hydrocarbon contaminants: an overview. Biotechnology research international 2010; 2011.

[17] Dash RR, Gaur A, Balomajumder C. Cyanide in industrial wastewaters and its removal: A review on biotreatment. Journal of Hazardous Materials 2009; 163(1) 1-11.

[18] Dubey SK, Holmes DS. Biological cyanide destruction mediated by microorganisms. World Journal of Microbiology and Biotechnology 1995; 11(3) 257-265.

[19] Dumestre A, Chone T, Portal J, Gerard M, Berthelin J. Cyanide degradation under alkaline conditions by a strain of *Fusarium solani* isolated from contaminated soils. Applied and Environmental Microbiology 1997; 63(7) 2729-2734.

[20] Dursun AY, Aksu Z. Biodegradation kinetics of ferrous(II) cyanide complex ions by immobilized *Pseudomonas fluorescens* in a packed bed column reactor. Process Biochemistry 2000; 35(6) 615-622.

[21] Dursun AY, Çalık A, Aksu Z. Degradation of ferrous(II) cyanide complex ions by *Pseudomonas fluorescens*. Process Biochemistry 1999; 34(9) 901-908.

[22] EPA U (1999). Integrated Risk Information System (IRIS), EPA.

[23] EPA. (1994). Treatment of cyanide heap leaches and tailings. EPA/530/R-94/037. USA.

[24] Ezzi MI, Lynch JM. Biodegradation of cyanide by *Trichoderma sp.* and *Fusarium sp.* Enzyme and Microbial Technology 2005; 36(7) 849-854.

[25] FAO (2014). "Food wastage: Key facts and figures." from http://www.fao.org/news/story/en/item/196402/icode/.

[26] Figueira MM, Ciminelli VST, Andrade MCd, Linardi VR. Cyanide degradation by an *Escherichia coli* strain. Canadian Journal of Microbiology 1996; 42(5) 519-523.

[27] Fry WE, Millar RL. Cyanide degradion by an enzyme from Stemphylium loti. Archives of Biochemistry and Biophysics 1972; 151(2) 468-474.

[28] Gong J-S, Lu Z-M, Li H, Shi J-S, Zhou Z-M, Xu Z-H. Nitrilases in nitrile biocatalysis: recent progress and forthcoming research. Microbial Cell Factories 2012; 11 142.

[29] Gu S, Wang S, Yang Q, Yang P, Peng Y. Start up partial nitrification at low temperature with a real-time control strategy based on blower frequency and pH. Bioresource technology 2012; 112(0) 34-41.

[30] Gullicks H, Hasan H, Das D, Moretti C, Hung Y-T. Biofilm fixed film systems. Water 2011; 3(3) 843-868.

[31] Gupta N, Balomajumder C, Agarwal V. Enzymatic mechanism and biochemistry for cyanide degradation: a review. Journal of Hazardous Materials 2010; 176(1) 1-13.

[32] Gupta V, Nayak A. Cadmium removal and recovery from aqueous solutions by novel adsorbents prepared from orange peel and Fe_2O_3 nanoparticles. Chemical Engineering Journal 2012; 180 81-90.

[33] Gurbuz F, Ciftci H, Akcil A. Biodegradation of cyanide containing effluents by Scenedesmus obliquus. Journal of Hazardous Materials 2009; 162(1) 74-79.

[34] Kao CM, Liu JK, Lou HR, Lin CS, Chen SC. Biotransformation of cyanide to methane and ammonium-nitrogen by Klebsiella oxytoca. Chemosphere 2003; 50(8) 1055-1061.

[35] Kowalska M, Bodzek M, Bohdziewicz J. Biodegradation of phenols and cyanides using membranes with immobilized microorganisms. Process Biochemistry 1998; 33(2) 189-197.

[36] Kwon HK, Woo SH, Park JM. Degradation of tetracyanonickelate (II) by *Cryptococcus humicolus* MCN2. FEMS Microbiology Letters 2002; 214(2) 211-216.

[37] Liang S, Guo X-Y, Feng N-C, Tian Q-H. Effective removal of heavy metals from aqueous solutions by orange peel xanthate. Transactions of Nonferrous Metals Society of China 2010; 20 s187-s191.

[38] Luque-Almagro V, Blasco R, Huertas M, Martínez-Luque M, Moreno-Vivián C, Castillo F, Roldán M. Alkaline cyanide biodegradation by *Pseudomonas pseudoalcaligenes* CECT5344. Biochemical Society Transactions 2005; 33(Pt 1) 168-169.

[39] Makkar R, Cameotra S. An update on the use of unconventional substrates for biosurfactant production and their new applications. Applied Microbiology and Biotechnology 2002; 58(4) 428-434.

[40] Makkar RS, Cameotra SS, Banat IM. Advances in utilization of renewable substrates for biosurfactant production. AMB Express 2011; 1(5) 1-19.

[41] Maniyam MN, Sjahrir F, Ibrahim AL. Bioremediation of cyanide by optimized resting cells of Rhodococcus strains isolated from Peninsular Malaysia. International Journal of Bioscience Biochemistry and Bioinformatics 2011; 1 98-102.

[42] Mekuto L, Jackson VA, Ntwampe SKO. Biodegradation of free cyanide using *Bacillus* sp. consortium dominated by *Bacillus safensis*, *Lichenformis* and *Tequilensis* strains: A bioprocess supported solely with whey. Bioremediation and Biodegradation 2013; s18(004) 1-7.

[43] Mitchell DA, Berovič M, Krieger N (2006). *Solid-state fermentation bioreactor fundamentals: introduction and overview*, Springer.

[44] Montgomery DC (2008). *Design and analysis of experiments*, John Wiley & Sons.

[45] Mulligan CN. Environmental applications for biosurfactants. Environmental Pollution 2005; 133(2) 183-198.

[46] Mussatto SI, Ballesteros LF, Martins S, Teixeira JA. Use of Agro-industrial waste in solid-state fermentation processes. Industrial waste. Croatia: InTech 2012; 121-140.

[47] Myers RH, Montgomery DC, Anderson-Cook CM (2009). *Response surface methodology: process and product optimization using designed experiments*, John Wiley & Sons.

[48] Nazly N, Knowles CJ, Beardsmore AJ, Naylor WT, Corcoran EG. Detoxification of cyanide by immobilised fungi. Journal of Chemical Technology and Biotechnology 1983; 33(2) 119-126.

[49] Negi R, Satpathy G, Tyagi YK, Gupta RK. Biosorption of heavy metals by utilising onion and garlic waste. International Journal of Environment and Pollution 2012; 49(3) 179-196.

[50] Nolan LM, Harnedy PA, Turner P, Hearne AB, O'Reilly C. The cyanide hydratase enzyme of *Fusarium lateritium* also has nitrilase activity. FEMS Microbiology Letters 2003; 221(2) 161-165.

[51] Ntwampe SK, Santos BA. Potential of agro-waste extracts as supplements for the continous bioremediation of free cyanide contaminated wastewater. International Journal of Agricultural, Biosystems Science and Engineering 2013; 7(7) 285- 289.

[52] O'Reilly C, Turner PD. The nitrilase family of CN hydrolysing enzymes – a comparative study. Journal of Applied Microbiology 2003; 95(6) 1161-1174.

[53] Oelofse SH, Nahman A. Estimating the magnitude of food waste generated in South Africa. Waste Management and Research 2013; 31(1) 80-86.

[54] Ojumu TV, Solomon BO, Betiku E, Layokun SK, Amigun B. Cellulase Production by Aspergillus flavus Linn Isolate NSPR 101 fermented in sawdust, bagasse and corncob. African Journal of Biotechnology 2003; 2(6) 150-152.

[55] Osuntokun BO (1994). Chronic cyanide intoxication of dietary origin and a degenerative neuropathy in Nigerians. International Workshop on Cassava Safety 375.

[56] Patil Y, Paknikar K. Development of a process for biodetoxification of metal cyanides from waste waters. Process Biochemistry 2000; 35(10) 1139-1151.

[57] Pereira PT, Arrabaça JD, Amaral-Collaço MT. Isolation, selection and characterization of a cyanide-degrading fungus from an industrial effluent. International Biodeterioration and Biodegradation 1996; 37(1–2) 45-52.

[58] Potivichayanon S, Kitleartpornpairoat R. Biodegradation of Cyanide by a Novel Cyanide-degrading Bacterium. Biodegradation 2014; 1 1362-1365.

[59] Rao M, Scelza R, Scotti R, Gianfreda L. Role of enzymes in the remediation of polluted environments. Journal of Soil Science and Plant Nutrition 2010; 10(3) 333-353.

[60] Rodrigues L, Teixeira J, Oliveira R. Low-cost fermentative medium for biosurfactant production by probiotic bacteria. Biochemical Engineering Journal 2006; 32(3) 135-142.

[61] Santos BAQ Continous bioremediation of electroplating effluent. Unpubished MTech thesis, Cape Peninsula University of Technology, Cape Town, South Africa 2013; 98.

[62] Santos BAQ, Ntwampe SKO, Doughari JH. Continuous biotechnological treatment of cyanide contaminated waters by using a cyanide resistant species of Aspergillus

awamori. Environmental Biotechnology–New Approaches and Prospective Applications, M. Petre, Ed. Croatia: InTech 2013; 123-146.

[63] Silva-Avalos J, Richmond MG, Nagappan O, Kunz DA. Degradation of the metal-cyano complex tetracyanonickelate (II) by cyanide-utilizing bacterial isolates. Applied and Environmental Microbiology 1990; 56(12) 3664-3670.

[64] Skowronski B, Strobel GA. Cyanide resistance and cyanide utilization by a strain of *Bacillus pumilus*. Canadian Journal of Microbiology 1969; 15(1) 93-98.

[65] Sobrinho HBdS, Luna JMd, Rufino RD, Porto ALF, Sarubbo LA. Assessment of toxicity of a biosurfactant from Candida sphaerica UCP 0995 cultivated with industrial residues in a bioreactor. Electronic Journal of Biotechnology 2013; 16(4) 4-4.

[66] Suh Y-J, Park JM, Yang J-W. Biodegradation of cyanide compounds by *Pseudomonas fluorescens* immobilized on zeolite. Enzyme and Microbial Technology 1994; 16(6) 529-533.

[67] Van Hamme JD, Singh A, Ward OP. Recent advances in petroleum microbiology. Microbiology and Molecular Biology Reviews 2003; 67(4) 503-549.

[68] Watanabe A, Yano K, Ikebukuro K, Karube I. Cyanide hydrolysis in a cyanide-degrading bacterium, *Pseudomonas stutzeri* AK61, by cyanidase. Microbiology 1998; 144(6) 1677-1682.

[69] Way JL. Cyanide intoxication and its mechanism of antagonism. Annual Review of Pharmacology and Toxicology 1984; 24(1) 451-481.

[70] White DM, Schnabel W. Treatment of cyanide waste in a sequencing batch biofilm reactor. Water Research 1998; 32(1) 254-257.

[71] Zhu Z, Zhang F, Wei Z, Ran W, Shen Q. The usage of rice straw as a major substrate for the production of surfactin by Bacillus amyloliquefaciens XZ-173 in solid-state fermentation. Journal of Environmental Management 2013; 127(0) 96-102.

[72] Zilouei H, Soares A, Murto M, Guieysse B, Mattiasson B. Influence of temperature on process efficiency and microbial community response during the biological removal of chlorophenols in a packed-bed bioreactor. Applied Microbiology and Biotechnology 2006; 72(3) 591-599.

[73] Zou S, Yao S, Ni J. High-efficient nitrogen removal by coupling enriched autotrophic-nitrification and aerobic-denitrification consortiums at cold temperature. Bioresource Technology 2014; 161 288-296.