

RESEARCH PAPER

Sequential biodegradation and sodium percarbonate oxidation for the removal of free cyanide from the liquid phase of gold plant tailings

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ABSTRACT

Hydrometallurgical gold processing generates cyanide-bearing solutions that must be treated to low residual levels with moderate reagent demand. This work evaluated the sequential use of biological degradation and chemical post-oxidation for the liquid phase of a gold plant tailings storage facility containing 443.2 mg/dm³ of free cyanide. The biological stage was carried out with an indigenous cyanide-degrading strain isolated from the same solution and identified by 16S rRNA gene sequencing as *Janibacter hoylei* C4; sodium percarbonate was used as the oxidant. Operating parameters were established for each stage, after which both stages were combined in a single reactor without intermediate separation of the biomass. When the optimized biological treatment was applied to the actual industrial tailings solution, the free cyanide concentration decreased from 443.2 to 101.9 mg/dm³, corresponding to a degree of detoxification of 77.0%. The addition of 1.35 g/dm³ of sodium percarbonate reduced the residual content to 0.04 mg/dm³, with an overall detoxification degree of 99.99%. The chemical treatment, applied on its own, reached 87.33% at a dosage of 3.5 g/dm³. The biological stage removed the bulk of the cyanide load, whereas the chemical stage brought the solution to the discharge limit, so the sequential scheme consumed 2.6 times less oxidant than the single-stage chemical route.

Keywords: free cyanide; tailings solution; biodegradation; *Janibacter hoylei*; sodium percarbonate; detoxification

INTRODUCTION

Cyanide leaching remains the principal hydrometallurgical route for gold recovery. The reagent is selective for precious metals and yields high recoveries from ores of different types. Alternative lixiviant systems based on thiosulfate, thiourea and glycine continue to develop, yet sodium cyanide is still applied at most gold recovery plants. Cyanidation generates large volumes of liquid waste containing various cyanide compounds, and these solutions must be treated before the water is returned to the circuit or discharged into the environment [1-4].

The liquid phase of a tailings storage facility is more complex than the model solutions normally used in laboratory studies. Besides free cyanide it contains thiocyanate, cyanate, stable metal-cyanide complexes, dissolved heavy metals and sulfur compounds. The individual cyanide species differ in stability and in the mechanisms of their destruction, so results obtained on model solutions do not transfer to industrial effluents without reservations [5-9].

Chemical, physicochemical, electrochemical and biological methods have been proposed for the treatment of cyanide-bearing solutions [10]. Chemical oxidation, including the SO₂/air (INCO) process and treatment with hydrogen peroxide, Caro's acid, or ozone, rapidly destroys free cyanide but consumes large amounts of reagent and loses efficiency in complex industrial solutions. Biological methods are cheaper to operate and more environmentally safe, although the process is slow and the microorganisms are sensitive to effluent composition and to the concentration of toxic components. Taken separately, neither approach fully covers the task [11-15].

Research has therefore shifted from the search for a universal method towards sequential treatment schemes. The combination of biological degradation and chemical oxidation appears most promising: a biological pre-treatment lowers the solution's toxicity and reduces the load on the chemical stage. In most published studies the two treatments are examined as independent processes and the distribution of the load between them is not analysed [16]. A considerable part of this work was performed on model solutions, whereas data on real tailings solutions remain scarce. Indigenous cyanide-degrading microorganisms and sodium percarbonate have received little attention as components of sequential schemes [17].

The present work evaluated the sequential use of a biological and a chemical stage as a single process in which each stage performs its own technological function. The working assumption was that biological removal of free cyanide would reduce the chemical load on the oxidation stage, reduce sodium percarbonate consumption, and provide the required treatment depth without complicating the flowsheet.

The object of the study was the cyanide-bearing liquid phase of an operating tailings storage facility. An indigenous cyanide-degrading strain isolated from the same solution served as the biological agent and sodium percarbonate as the oxidant. The operating parameters of each stage, the distribution of the load between them and the performance of the scheme on the industrial solution were determined [18].

MATERIAL AND METHODS

Sampling and physicochemical characterisation of the tailings solution

The cyanide-bearing liquid phase of the tailings storage facility at the Altyntau Kokshetau gold recovery plant (Akmola region, Republic of Kazakhstan) was used as the study object. The solution is formed after cyanidation and sorption recovery of gold; its chemical composition is typical of industrial cyanide-bearing effluents and includes free cyanide, thiocyanate, metal-cyanide complexes, dissolved metals and sulfur compounds.

Samples were taken from several points of the facility in accordance with ISO 5667-3:2018, placed in sterile high-density polyethylene containers, transported to the laboratory at (4 ± 2) °C and analysed no later than 48 h after sampling. This regime limits changes in the chemical composition and in the microbiological characteristics of the sample.

The pH, oxidation-reduction potential (ORP), temperature and electrical conductivity of the solution were measured before the experiments. The chemical composition was monitored before treatment and after each stage. The initial composition of the liquid phase is given in **Table 1**.

Dissolved metals were determined by inductively coupled plasma optical emission spectrometry (ICP-OES, Optima 8300DV, PerkinElmer, USA). Free cyanide was determined spectrophotometrically with Spectroquant test kits (Merck, Germany)

and the readings were verified with a cyanide-selective electrode. Thiocyanate and ammonium were determined photometrically. Functional groups and metal-cyanide complexes were identified by Fourier transform infrared spectroscopy

(FTIR, FT/IR-6X, JASCO, Japan) with an attenuated total reflectance (ATR) accessory.

Table 1 Chemical composition of the liquid phase of the sorption leaching tailings of the Altyntau Kokshetau gold recovery plant

Component	Au	Cu	As	Fe	Ag	Zn	S(total)	S ²⁻	CN ⁻ (free)	SCN ⁻
Concentration, mg/dm ³	0.101	4.74	7.0	3.7	0.1	0.6	658.2	246.7	443.2	257.5

Isolation and identification of the indigenous cyanide-degrading strain

The biological stage was performed using indigenous microorganisms isolated from the cyanide-bearing liquid phase of the Altyntau Kokshetau tailings storage facility. Populations that persist for long periods at elevated concentrations of cyanide and dissolved metals are adapted to these conditions, which determined the choice of the inoculum source.

Samples of the liquid phase were cultivated in a mineral medium in which the cyanide-bearing solution served as the nitrogen source. After enrichment culturing the cultures were plated on solid media and single bacterial colonies were obtained. Pure cultures C1 to C5 were isolated by successive subculturing.

Growth of the isolates in the presence of cyanide was followed by the optical density of the bacterial suspension (OD₆₀₀) and by the decrease in the free cyanide concentration in the culture medium (**Table 2**). Strain C4 was selected for further work because it showed stable growth in the cyanide-bearing medium and reproducible detoxification results.

Cell morphology was characterised by Gram staining and standard biochemical tests. The species was identified by sequencing of the 16S rRNA gene. Genomic DNA was extracted by the CTAB method, amplification was performed with the

universal primers 8F and 806R, and the PCR products were sequenced by the Sanger method.

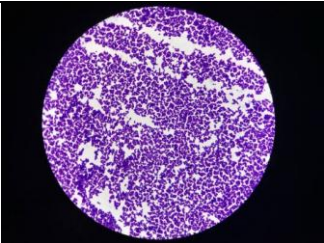
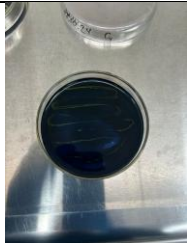
Table 2 Optical density and pH of the bacterial samples in the presence and in the absence of sodium cyanide

No	Strain	OD ₆₀₀ , NaCN +	OD ₆₀₀ , NaCN –	pH, NaCN +	pH, NaCN –
1	C1	0.532	0.498	9.21	8.52
2	C2	0.563	0.545	9.86	7.12
3	C3	0.589	0.465	9.41	7.69
4	C4	0.521	0.489	9.32	8.22
5	C5	0.567	0.543	9.56	8.37

The nucleotide sequences were compared with the NCBI GenBank database using BLAST. The strain was assigned to the species *Janibacter hoylei*.

The main characteristics of the isolate are given in **Table 3**. *Janibacter hoylei* C4 was used as the biological agent in the first stage of the treatment of the cyanide-bearing liquid phase.

Table 3 Characteristics and molecular identification of the selected strain

Strain	Gram staining	Catalase	Amylase
C4			
	+ cocci	+	–

Sequential biological and chemical treatment

The treatment scheme comprised two consecutive stages: biological degradation of free cyanide and post-oxidation of the residual cyanide compounds with sodium percarbonate. The experimental flowsheet is shown in **Fig. 1**.

Before the biological treatment the raw solution was filtered through a membrane filter with a pore size of 0.45 µm, which removed suspended solids and preserved the dissolved cyanide species and the original physicochemical characteristics of the liquid phase. The filtrate was used in all subsequent experiments.

In the first stage the solution was inoculated with the isolated indigenous strain and incubated under the conditions established in preliminary tests. The purpose of the stage was to reduce the free cyanide concentration and the toxic load for subsequent chemical oxidation.

The bacterial biomass was not separated by stage. After the biological stage the reaction mixture was passed to chemical post-oxidation without additional filtration. This excludes the phase separation operations, shortens the flowsheet and allows the whole process to be run in a single reaction volume.

Sodium percarbonate (2Na₂CO₃·3H₂O₂) was introduced into the reaction system in the second stage. Post-oxidation was performed at different reagent dosages, treatment times, pH values, and temperatures in accordance with the experimental plan. The stage was intended to remove the free cyanide remaining after the biological treatment.

The performance of the scheme was evaluated by comparing the biological stage, the chemical post-oxidation and their sequential application. The criteria were the residual free cyanide concentration, the degree of detoxification, the thiocyanate content, the pH and ORP values, and the consumption of sodium percarbonate relative to the stand-alone chemical treatment.

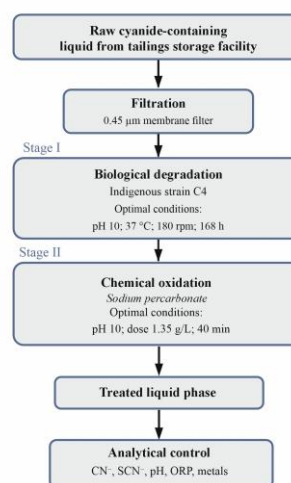


Fig. 1 Sequential scheme of biological and chemical treatment

Analytical methods

The efficiency of the sequential treatment was evaluated from the change in the physicochemical characteristics of the solution before treatment, after the biological stage and after the chemical post-oxidation. The residual free cyanide concentration, the detoxification degree, the thiocyanate content, the concentrations of dissolved metals and the pH and ORP values were taken into account.

Free cyanide was determined spectrophotometrically with Spectroquant test kits (Merck, Germany) on a Spectroquant Prove 100 VIS spectrophotometer, and the measurements were duplicated with a cyanide-selective electrode. Thiocyanate and ammonium were determined photometrically following the procedures of the test kit manufacturer.

Dissolved metals (Au, Ag, Cu, Fe, Zn, As) were determined by ICP-OES (Optima 8300DV, PerkinElmer, USA); for individual elements the results were confirmed by flame atomic absorption spectrometry (AAS). The change in the composition of the cyanide compounds before and after treatment was followed by FTIR spectroscopy (FT/IR-6X, JASCO, Japan) with an ATR accessory.

The pH and ORP values were monitored throughout the experiment in both stages of the treatment.

Experimental reproducibility and analytical quality control

The reproducibility of the biological treatment at the central optimized condition (pH 10.0, 37 °C, 180 rpm and 168 h) was evaluated in three independent experiments ($n = 3$). The results are expressed as mean $\pm$ standard deviation (SD). Under these conditions, the mean OD₆₀₀ and free-cyanide detoxification efficiency were 1.10 ± 0.09 and $80 \pm 4\%$, respectively.

ICP-OES measurements were performed in triplicate, with a relative analytical error not exceeding 3%. Free-cyanide concentrations determined spectrophotometrically were independently verified using a cyanide-selective electrode.

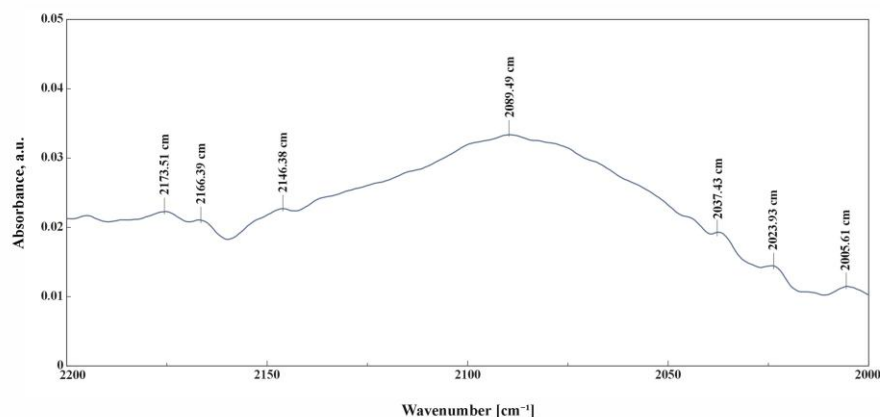


Fig. 2 Expanded fragment of the FTIR spectrum of the raw tailings solution in the range 2200 to 2000 cm^{-1}

Selection of the indigenous cyanide-degrading strain

The biological stage requires microorganisms that retain metabolic activity at elevated concentrations of cyanide and dissolved metals, so the cultures were isolated directly from the tailings solution under study [20].

The optical densities of the isolated cultures differed only slightly (0.521 to 0.589), so the selection was based on the stability of growth in the cyanide-bearing medium and on the reproducibility of the detoxification results (Table 2). Strain C4 met these requirements. Retention of physiological activity in a medium with a composition close to that of the tailings solution was also taken into account.

Sequencing of the 16S rRNA gene assigned the selected strain to the species *Janibacter hoylei*. The morphological, biochemical and molecular genetic characteristics are given in the Material and methods section.

Janibacter hoylei C4, adapted to prolonged exposure to cyanide and dissolved metals, was used as the biological agent in the first stage of the sequential scheme. Its use provided stable operation of the biological stage on the industrial solution.

Biological stage as a pre-treatment step

The biological stage lowers the free cyanide concentration and the overall toxicity of the medium before the chemical oxidation. Compliance with the discharge limit was not expected from this stage: its task was to remove the bulk of the free cyanide and to prepare the solution for treatment with a smaller amount of oxidant.

RESULTS AND DISCUSSION

Physicochemical characterisation of the tailings solution

The composition of the liquid phase governs the conditions for both the microorganisms and the oxidant, so the solution was characterised before the treatment [19]. Tailings solutions are multicomponent systems in which free cyanide coexists with thiocyanate, metal-cyanide complexes, dissolved metals and sulfur compounds.

The solution studied contained 443.2 mg/dm^3 of free cyanide and 257.5 mg/dm^3 of thiocyanate, together with dissolved gold, silver, copper, iron, zinc, arsenic and sulfur compounds (Table 1). Such a composition is typical of the liquid phase of gold plant tailings facilities and indicates the simultaneous presence of free cyanide and stable metal-cyanide complexes, which differ in stability and in the mechanism of their destruction.

The FTIR analysis confirmed the presence of coordinated metal-cyanide complexes of copper, iron, zinc, silver, and gold (Fig. 2). Removal of free cyanide alone does not detoxify such a solution, so the treatment must act on the different cyanide species sequentially.

A free cyanide concentration of 443.2 mg/dm^3 suppresses cell respiration in most microorganisms. A biological stage on this solution is feasible only with cultures adapted to prolonged exposure to cyanide and dissolved metals.

The effect of the main factors on the activity of strain C4 was studied for the pH value, the cultivation temperature and the agitation intensity, since these parameters govern the growth of the microorganisms, the activity of the enzyme systems and the rate of cyanide transformation. The tests were run in a model system with an initial free cyanide concentration of 50 mg/dm^3 , which excluded the influence of the complex composition of the industrial water and allowed the physiological optimum of the microorganism to be determined [21].

Table 4 Operating conditions of the biological stage

Parameter	Range studied	Optimum value
pH	9.5 to 11.0	10.0
Temperature, °C	25 to 45	37
Agitation speed, rpm	100 to 200	180

Reproducibility at the central optimized condition (pH 10.0, 37 °C, 180 rpm, 168 h) was evaluated independently ($n = 3$), giving a mean detoxification efficiency of $80 \pm 4\%$.

The highest biological detoxification efficiency in the model system was observed at pH 10.0, a temperature of 37 °C and an agitation speed of 180 rpm (**Table 4, Fig. 3**). A detoxification efficiency of 82% was obtained in the corresponding optimization series. Reproducibility of the central optimized condition (pH 10.0, 37 °C, 180 rpm and 168 h) was additionally evaluated in three independent experiments, resulting in a mean free-cyanide detoxification efficiency of $80 \pm 4\%$ and an OD_{600} of 1.10 ± 0.09 ($n = 3$). Thus, the value of 82% observed in the optimization series lies within the experimental variability determined for the optimized condition.

It should be emphasized that the experiments presented in **Fig. 3** were performed in a model system containing an initial free cyanide concentration of 50 mg/dm³. These experiments were designed to establish the physiological operating optimum of strain C4 while minimizing the influence of the complex industrial matrix. The optimized conditions were subsequently evaluated using the actual industrial tailings solution containing 443.2 mg/dm³ of free cyanide.

Outside the optimum region the efficiency of the treatment decreased. A lower pH shifted the equilibrium between the cyanide ion and hydrogen cyanide, while alkalinity above the optimum suppressed the strain's physiological activity. Below 37 °C the enzymatic processes slowed down, and at higher temperatures inactivation of the enzyme systems began. Insufficient agitation limited mass transfer between the cells and the substrate, whereas excessive agitation could mechanically damage the biomass.

The 82% obtained was not the final target of the work. The biological treatment served as the first step: the decrease in free cyanide concentration reduced the toxic load at the chemical stage and created conditions for lower sodium percarbonate consumption. The parameters established here were used in the subsequent tests of the sequential scheme.

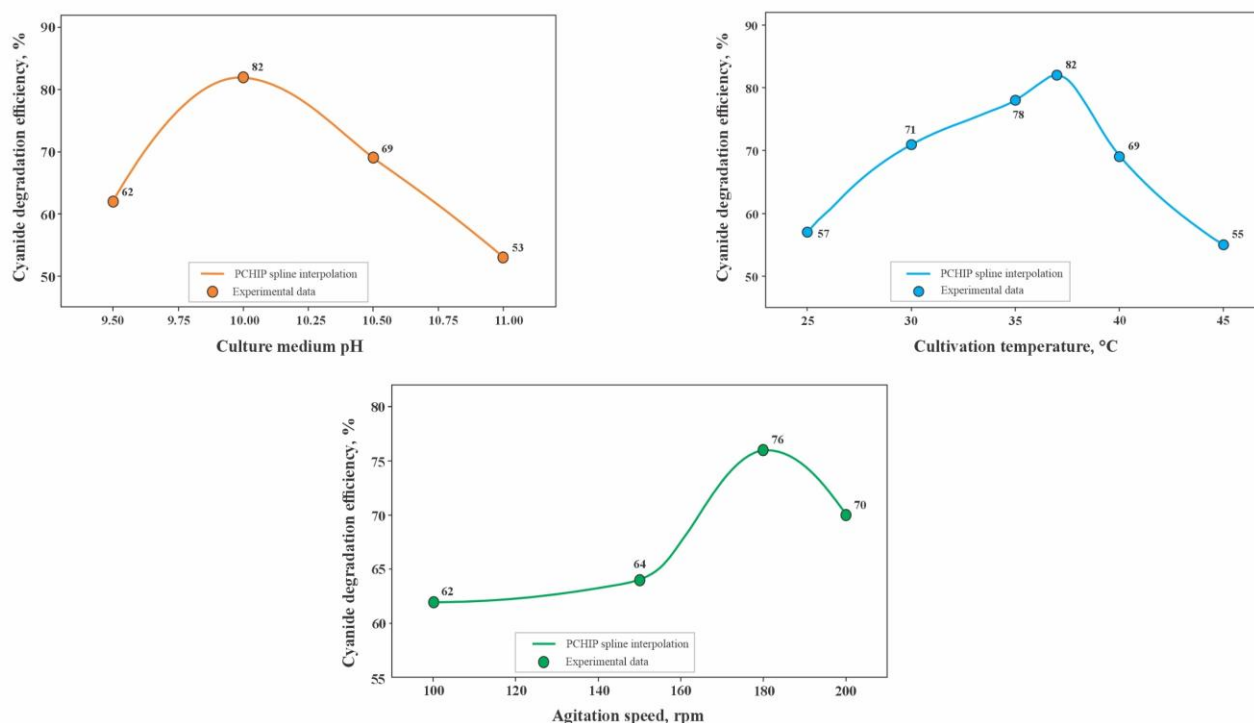


Fig. 3 Effect of pH, temperature and agitation intensity on the biological detoxification of free cyanide by strain C4 in the model system ($C_0(\text{CN}^-) = 50 \text{ mg/dm}^3$).

Chemical post-oxidation as the final stage

After the biological treatment the solution still contains free cyanide and more stable cyanide compounds that are not destroyed during the incubation time. They were removed by chemical post-oxidation with sodium percarbonate [22].

The oxidant was applied only to the solution that had passed the biological stage, so its consumption reflects the residual rather than the initial cyanide content.

The effects of the specific consumption of sodium percarbonate, the treatment time, and the pH value on the degree of detoxification of free cyanide were studied. The parameter ranges were set based on preliminary tests.

The degree of detoxification of free cyanide depended on the consumption of sodium percarbonate (**Fig. 4**). Efficiency increased with dosage, reaching a maximum of 87.33% at a consumption of 3.5 g/dm³. A higher percarbonate concentration yields a larger amount of active oxygen-bearing species that oxidise free cyanide [23].

The treatment time influenced the result to a similar extent. At the beginning of the reaction the detoxification rate remained low, and the degree of free cyanide oxidation increased with the contact time. The maximum was reached after 40 min, and a further increase in time is not justified technologically. The effect of the treatment time is more pronounced in the alkaline region, where the cyanide ion is stable.

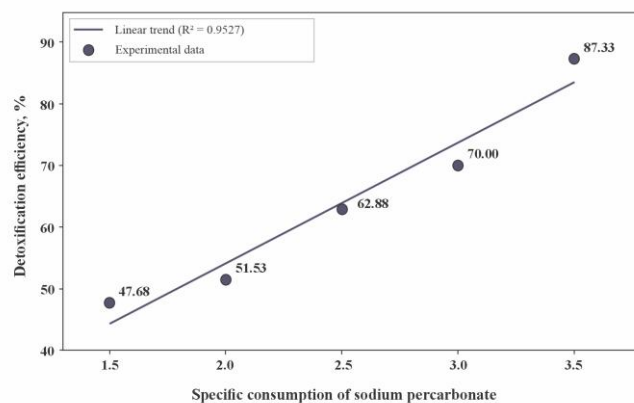


Fig. 4 Effect of the specific consumption of sodium percarbonate on the detoxification of free cyanide

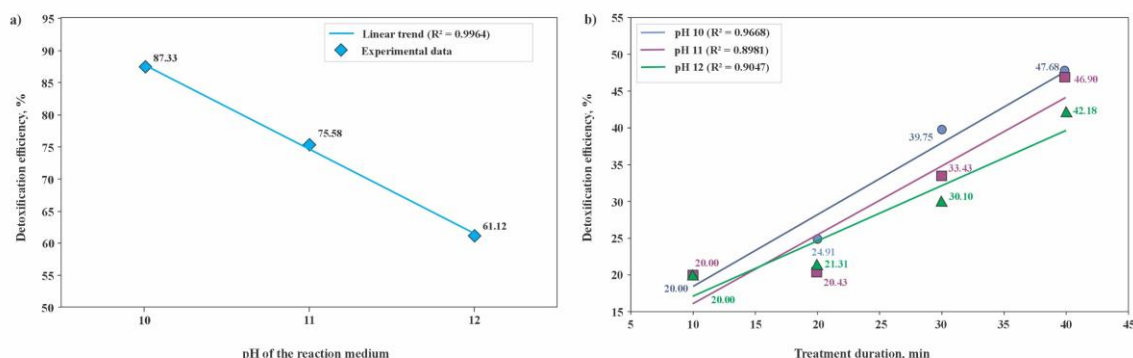
Table 5 Operating parameters of the chemical stage

Parameter	Value
Sodium percarbonate consumption, g/dm ³	3.5
pH	10
Treatment time, min	40
Temperature, °C	20 to 25
Agitation speed, rpm	300
Detoxification degree, %	87.33

The highest efficiency of the chemical treatment was obtained at pH 10 (Fig. 5), and a further increase in alkalinity reduced the degree of free cyanide oxidation. An optimum pH range therefore exists in which the formation of active oxygenen-

bearing species and their interaction with the cyanide ion proceed most completely. At higher pH values the mechanism of the radical reactions changes and the share of the most reactive oxidising forms decreases.

The operating parameters of the chemical stage were a sodium percarbonate consumption of 3.5 g/dm³, a treatment time of 40 min and pH 10 (Table 5). Under these conditions the oxidation of the residual cyanide proceeded with the highest efficiency, and these values were used in the evaluation of the sequential scheme. These parameters refer to the post-oxidation of a solution that has passed the biological treatment and not to a stand-alone chemical detoxification. The efficiency of the stage also depends on the state of the solution after the biological transformation, so the outcome was evaluated from the result of the sequential application of both stages.

**Fig. 5** Effect of the treatment time and of the pH value on the chemical detoxification of free cyanide

Sequential application of the biological and chemical stages

After the operating conditions of both stages had been established, their sequential application to the cyanide-bearing tailings solution was evaluated. The aim was to determine the contribution of each stage to the overall efficiency and to compare the scheme with the single-stage treatment options [24].

Both stages were carried out in a single reactor without intermediate separation of the bacterial biomass; sodium percarbonate was introduced directly into the reaction system after the biological treatment was completed. This removes the filtration, pumping, and biomass separation operations and reduces solution loss and the risk of secondary contamination.

During the biological stage the free cyanide concentration decreased from 443.2 to 101.9 mg/dm³, which corresponds to a detoxification degree of 77 %. This value should be distinguished from the maximum value of 82% obtained during optimization of the biological stage in the model system (Fig. 3). The optimization experiments were performed at an initial free cyanide concentration of 50 mg/dm³, whereas the sequential treatment was evaluated using the actual industrial tailings solution containing 443.2 mg/dm³ of free cyanide. In addition to the almost nine-fold higher initial free-cyanide concentration, the industrial solution contained thiocyanate, dissolved metals, sulfur compounds and metal-cyanide complexes. Therefore, the slightly lower biological detoxification efficiency of 77% reflects the transfer of the optimized conditions from the model system to a substantially more concentrated and chemically complex industrial matrix rather than an inconsistency between the experimental results. Biodegradation was most intensive during the first two days of incubation, after which the rate decreased and by the end of the seventh day the system reached a steady state. A longer biological treatment gave only a small additional decrease and was found technologically unjustified.

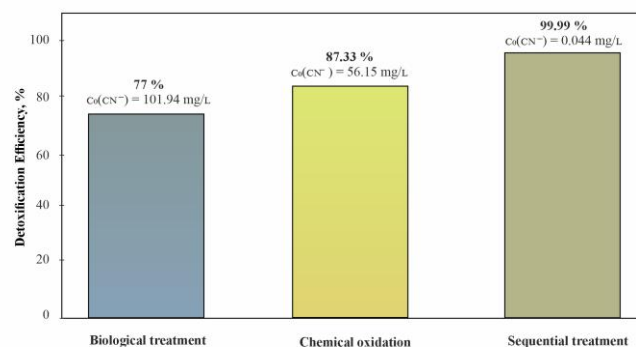
The addition of sodium percarbonate oxidised the residual free cyanide rapidly. As the reagent consumption increased, the overall efficiency of the process rose from 77.0 % to 99.99 % and the residual free cyanide concentration fell to 0.04 mg/dm³, which meets the limit set for fishery water bodies. A consumption of 1.35 g/dm³ was adopted for the sequential scheme.

The biological pre-treatment reduced the oxidant demand. In the stand-alone chemical treatment a consumption of 3.5 g/dm³ gave 87.33 %, whereas the

sequential scheme reached 99.99 % at 1.35 g/dm³, that is at a 2.6 times lower consumption of sodium percarbonate [25].

The biological stage converts the bulk of the free cyanide enzymatically and the chemical stage oxidises the residue that resists biodegradation.

Neither the biological nor the chemical stage on its own brought the solution to the required residual free cyanide concentration (Fig. 6). After the biological treatment the residual concentration remained well above the limit, and chemical oxidation without pre-treatment required a considerably higher reagent consumption. The limit was met only when both stages were applied in sequence [26].

**Fig. 6** Comparison of the biological, chemical and sequential treatment schemes

The two stages operate on different time scales (Fig. 7). The biological stage lowers the free cyanide concentration over several days through the enzymatic activity of the microorganisms, while the chemical stage destroys the residue within tens of minutes. This load distribution provides a high degree of detoxification with lower reagent consumption.

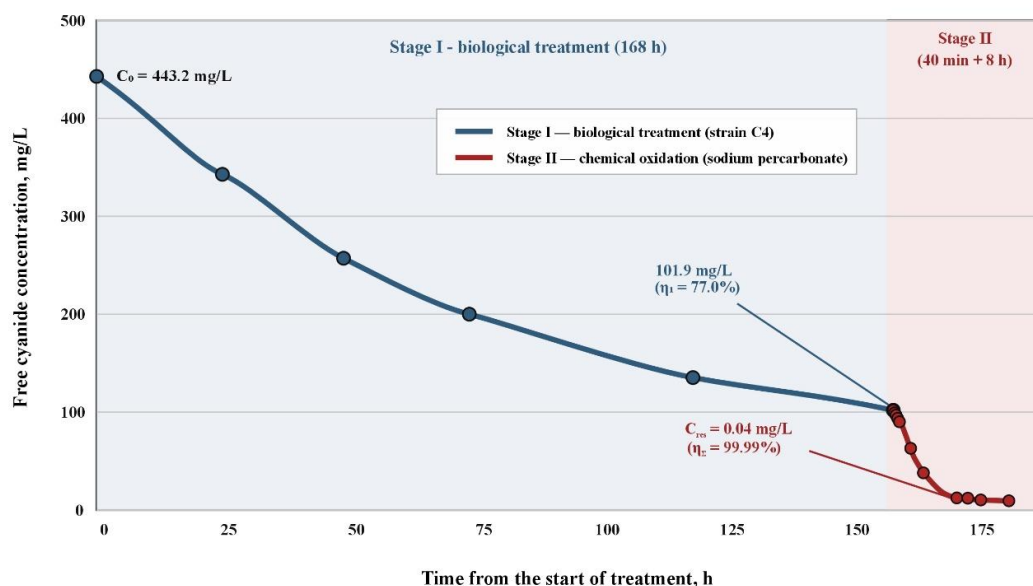


Fig. 7 Change in the free cyanide concentration during the sequential biological and chemical treatment

CONCLUSION

The sequential application of biological and chemical stages was evaluated for the cyanide-bearing liquid phase of a hydrometallurgical tailings storage facility. Biological treatment with the indigenous strain *Janibacter hoylei* C4 at pH 10.0, 37 °C and 180 rpm removed the main part of the free cyanide and lowered the toxic load on the chemical stage, while post-oxidation with sodium percarbonate at pH 10 and a treatment time of 40 min completed the destruction of the residual cyanide. Both stages were run in a single reactor without intermediate separation of the biomass, thereby keeping the flowsheet short.

In the sequential scheme the free cyanide concentration decreased from 443.2 to 0.04 mg/dm³ at an overall detoxification degree of 99.99 % and a sodium percarbonate consumption of 1.35 g/dm³, against 3.5 g/dm³ in the stand-alone chemical treatment, that is a 2.6 times lower reagent demand. The results support the use of biological degradation followed by chemical post-oxidation for the treatment of cyanide-bearing solutions of hydrometallurgical gold production. Further work should address scale-up, the stability of the scheme under a changing composition of the industrial solution and a techno-economic assessment covering reagent consumption, energy demand and the reuse of the treated water.

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