

# Semi-Global Output Feedback Stabilization from Non-Minimum Nonlinear Phase Systems

Ansar<sup>1\*</sup> and J Naiborhu<sup>2</sup>

<sup>1</sup>Program Studi Pendidikan Matematika Fakultas Keguruan dan Ilmu pendidikan Universitas Sembilanbelas November Kolaka , Jl. Pemuda No. 339, Kolaka, Indonesia.

<sup>2</sup>Program Studi Magister Matematika Fakultas Matematika dan Ilmu Pengetahuan Alam Institut Teknologi Bandung, Bandung, Indonesia

\*E-mail: ansarmawasangka@gmail.com

**Abstract.** Nickel is a valuable sediment material obtained by mining. The main problem in the nickel post-mining land at Pomalaa is the toxicity of nickel (Ni) and chromium (Cr). The results of the analysis of soil samples showed that nickel levels reached 11103.74 mg/kg and chromium reached 4030.17 mg/kg. The toxicity limit for plants for nickel is 100 ppm and chromium is 20 ppm. The toxicity of nickel and chromium can cause the degradation of soil and plant fertility. Bacteria are biological agents that are environmentally friendly which can be used to reduce metal toxicity on post-mining land. This study aims to examine biochemically 6 indigenous bacteria resistant to 10 ppm NiCl<sub>2</sub> and 4 indigenous bacteria resistant to 10 ppm CrCl<sub>2</sub> and identification. Biochemical tests were carried out namely gram test (3% KOH), catalase test (3% H<sub>2</sub>O<sub>2</sub>), oxidation-fermentation (O-F) test and endospores test. Based on the results of biochemical tests known indigenous bacteria from the genus *Bacillus* Sp. is gram-positive (+) bacteria that do not have the catalase enzyme, do not have the ability to use carbohydrates and produce endospores in their life cycle while indigenous bacteria from the genus *Clostridium* sp. is a gram-positive (+) bacterium which in part has the enzyme catalase, all of which have the ability to use carbohydrates and only some of them produce endospores.

## 1. Introduction

Pomalaa is a nickel producing sub-district in Kolaka Regency, Southeast Sulawesi, Indonesia. The management of nickel post-mining land at Pomalaa is revegetation, various types of plants are planted in post-mining land, but the revegetation effort is not enough to restore soil fertility. The results of the nutrient analysis of plants on revegetated land showed deficiencies of K, Ca, Fe, Cu, Mn [1]. The results of the analysis of heavy metals are known to occur in the toxicity of nickel (Ni) and Chromium (Cr) [1]. The toxicity limit for plants for nickel is 100 ppm and chromium is 20 ppm [2]. The limit of nickel metal content in soil, water, and the human body is 4-80 ppm [3]. Nickel and chromium are included in heavy metals whose availability in high concentrations of soil can be toxic so they can degrade soil fertility and inhibit plant growth. High concentrations of heavy metals will inhibit growth, change morphology and interfere with the metabolism of organisms *in vitro* [4].

Indigenous bacteria are bacteria isolated from their habitat and then cultured *in vitro*. One effort to reduce heavy metal toxicity and increase soil fertility on post-mining land is by utilizing indigenous bacteria in the mine. Bacteria in site habitats contaminated with heavy metals develop several

mechanisms of tolerance to heavy metals, most of the mechanisms of microbial tolerance to metals are by metal efflux outside the cell [5]. Bacteria isolated from an environment contaminated with heavy metals have resistance to heavy metals around them [6]. The use of bacteria to degrade polluted soil has been widely studied. *Flavobacterium*, *Pseudomonas* and *Azoarcus* sp carry out bioremediation of total petroleum hydrocarbons [7]. *Bacillus* sp. is capable of degrading toluene-contaminated soil [8]. *Bacillus* bacteria have a good ability to degrade heavy metal metals such as Cu, Fe, Zn, Ni, and Pb [9].

Biochemical tests and identification were carried out to characterize pure culture from isolation through its morphological and physiological properties. This study aims to examine biochemically six indigenous bacterial isolates that have been previously tested have the ability to grow and develop well in media added 10 ppm NiCl<sub>2</sub>, namely isolates R, Q, TLB1, TLB2, II, III and four indigenous bacterial isolates capable of growing and developing well on media added 10 ppm CrCl<sub>2</sub>, namely isolates G, TLB3, TLB4, and TTC. The bacterial isolate tested was a collection of Biotechnology Research and Development Center, Hasanuddin University, Makassar. The results of biochemical tests can be a recommendation in future studies to obtain potential bacteria as biological agents that can be used to reduce metal toxicity on post-mining land and increase soil fertility.

## 2. Methods

This study began with an analysis of soil samples where indigenous bacteria were isolated. Furthermore, the research is divided into two stages. The first stage is a gram test (3% KOH), and a biochemical characteristic test. Biochemical characteristic tests include catalase (3% H<sub>2</sub>O<sub>2</sub>), Oxidation-Fermentation (OF) test and endospores test. The second stage is morphological observation and identification.

### 2.1. Analysis of soil at the sampling location

Analysis of soil samples was carried out to obtain the latest data on the levels of nickel and chromium in the post-mining land where indigenous bacteria were isolated. Soil samples were taken at depths of 0-30cm, 30-60cm and 60-90cm at eleven mining locations, namely locations G, R, S, Q, TLB, TTC, TLE, Virgin, I, II, III. The soil sample is then composite. Furthermore, soil samples were analyzed at the Integrated Chemistry Laboratory of the Bogor Agricultural University. Analysis of soil samples using the United States Environment Protection Agency (USEPA) method: 7000B (1996).

### 2.2. Gram test (KOH 3%)

Gram test method with 3% KOH [10]. The gram test is carried out by taking one bacterial isolate and then mixing it with 2 drops of 3% KOH solution on top of the object glass, stirring circularly for 5-10 seconds with one needle, observed the formation of mucus. If mucus is formed on top of the glass the object indicates the isolate is a gram-negative bacterium, if it is not slimy it indicates gram-positive bacteria.

### 2.3. Catalase test (H<sub>2</sub>O<sub>2</sub> 3%)

Catalase test method with (3% H<sub>2</sub>O<sub>2</sub>) [11]. The H<sub>2</sub>O<sub>2</sub> test was carried out by piping 100μ of 30% H<sub>2</sub>O<sub>2</sub> in the object's glass, taken by one bacterial isolate and then streaked on the object's glass, observing the formation of bubbles. if there are bubbles indicating positive catalase bacteria if there are no bubbles including negative catalase bacteria [12].

### 2.4. Oxidation - Fermentation test (O-F)

The oxidation-fermentation test is carried out to determine the ability of bacteria to metabolize. Oxidation-Fermentation (O-F) test uses Hugh Leifson's media [13]. Hugh Leifson's media which was made into the test tube as much as 9 ml, the media was then autoclaved, cooled, 10% sterile glucose was added. Obtained by one bacterial isolate and then inoculated into the media, one bacterial isolate was inoculated on two tubes, fermentative bacteria will produce an acidic reaction on the closed or no tubes. Whereas oxidative bacteria will only produce acidic reactions in tubes that are not closed and a

little acid formation in the closed tube. The media was incubated for 2 days and then observed the color change. The acid produced from fermentation will reduce the pH of the media so that the *bromothymol blue* indicator becomes yellow [14].

### 2.5. Endospores test

Endospores test is done by coloring. Endospores test is carried out to determine the ability of bacteria to produce endospores. The fixed preparations are placed on a water bath then covered with filter paper. *Green malachite* drops are dropped and left for 5 minutes. The preparation is then washed with running water. Then re-color with *safranin* then leave for 60 seconds. The preparation was washed and then observed under a microscope. Positive test if the endospores are green while vegetative cells are red [15].

### 2.6. Identification

Identification was carried out to determine the genus of indigenous bacterial isolates tested. Identification is done through characterization observations of bacterial morphology including pigmentation, colony form, colony margin, and elevation.

## 3. Results and Discussions

### 3.1. Analysis of soil at the sampling location

Soil analysis was carried out using the USEPA method: 7000B (1996). Analysis of soil samples at the eleven sampling locations showed the highest nickel content reached 11103.74 mg/kg in sample III and the highest chromium content reached 4030.17 mg/kg in sample S. Based on the results of this analysis it can be concluded that nickel and chromium levels in nickel post-mining land at Pomalaa far above the threshold. Nickel and chromium are forms of heavy metals whose solubility in the soil can be toxic to plants and the environment. The results of the analysis of soil samples from the nickel post-mining land at Pomalaa are presented in Table 1.

**Table 1.** Analysis of soil from the nickel post-mining land at Pomalaa

Sample	Laboratory No.	Result**		Unit	Method
		Nickle, Ni	Chromium, Cr		
G	BM/VII/17/1638	10783.85	2068.54	mg/Kg	USEPA: 7000B (1996)
R	BM/VII/17/1636	2844.59	2419.46	mg/Kg	USEPA: 7000B (1996)
S	BM/VII/17/1637	5334.88	3630.11	mg/Kg	USEPA: 7000B (1996)
Q	BM/VII/17/1640	5497.92	2763.27	mg/Kg	USEPA: 7000B (1996)
TLB	BM/VII/17/1641	6186.43	3654.93	mg/Kg	USEPA: 7000B (1996)
TTC	BM/VII/17/1642	9130.32	2460.36	mg/Kg	USEPA: 7000B (1996)
TLE	BM/VII/17/1643	9606.37	3693.67	mg/Kg	USEPA: 7000B (1996)
virgin	BM/VII/17/1644	8986.82	4030.17	mg/Kg	USEPA: 7000B (1996)
I	BM/VII/17/1645	5613.10	3164.37	mg/Kg	USEPA: 7000B (1996)
II	BM/VII/17/1646	6402.34	2143.66	mg/Kg	USEPA: 7000B (1996)
III	BM/VII/17/1647	11103.74	3171.10	mg/Kg	USEPA: 7000B (1996)

\*) Outside the scope of accreditation

\*\*\*) Dry Basis

### 3.2. Gram test (KOH 3%) and catalase test (H<sub>2</sub>O<sub>2</sub> 3%)

Gram tests are used to distinguish gram-positive and gram-negative bacteria. The 10 indigenous bacterial isolates that were tested, all of them were gram-positive (+) bacteria which were characterized by the absence of mucus formed on the object of observation. Catalase test using 3% H<sub>2</sub>O<sub>2</sub> was carried out to determine the presence or absence of the catalase enzyme in bacteria. Catalase

is an enzyme that catalyzes the decomposition of hydrogen peroxide into water and O<sub>2</sub>. Hydrogen peroxide is formed during aerobic metabolism, so microorganisms that grow in an aerobic environment can emit these toxic substances. The catalytic test uses 3% H<sub>2</sub>O<sub>2</sub> because H<sub>2</sub>O<sub>2</sub> is one of the components produced by bacteria during the aerobic respiration process. Gram test results and catalase tests on 10 indigenous bacterial isolates varied results. Gram test and catalase test on 10 indigenous bacterial isolates are presented in Table 2.

**Table 2.** Gram test and catalase test on 10 indigenous bacterial isolates

Isolates	Gram test (KOH 3%)		Catalase test (H <sub>2</sub> O <sub>2</sub> 3%)	
	Observation	Results	Observation	Results
R	mucus is not formed	+	there are bubbles	+
Q	mucus is not formed	+	there are no bubbles	-
TLB1	mucus is not formed	+	there are no bubbles	-
TLB2	mucus is not formed	+	there are no bubbles	-
II	mucus is not formed	+	there are bubbles	+
III	mucus is not formed	+	there are bubbles	+
G	mucus is not formed	+	there are no bubbles	-
TLB3	mucus is not formed	+	there are no bubbles	-
TLB4	mucus is not formed	+	there are bubbles	+
TTC	mucus is not formed	+	there are bubbles	+

### 3.3. Oxidation - Fermentation (O-F) test and endospores test

Oxidation-Fermentation (O-F) are two important processes in the metabolism of microorganisms. O-F test is carried out to determine the level of ability of microorganisms to use carbohydrates while endospores are carried out to distinguish bacterial spores from vegetative cells. *Clostridium*, *Desulfomaculatum*, and *Bacillus* are bacteria that produce endospores in their life cycle. Endospores is a dormant form of vegetative cells, so its metabolism is inactive and is able to withstand environmental stresses such as heat, dryness, cold, radiation and pollutants. The results of O-F and endospores tests on 10 indigenous bacterial isolates showed mixed results. The O-F and endospores tests on 10 indigenous bacterial isolates are presented in Table 3.

**Table 3.** Oxidation – Fermentation(O-F) test and endospores test on 10 indigenous bacterial isolates

Isolates	O-F test		Endospores test
	Hasil		Results
	Oxidation	Fermentation	
R	+	+	-
Q	+	+	+
TLB1	-	-	+
TLB2	+	+	+
II	+	+	-
III	+	+	+
G	+	+	+
TLB3	+	+	+
TLB4	+	+	+
TTC	+	+	-

### 3.4. Identification

Identification was carried out to determine the genus of indigenous bacterial isolates tested. Based on the results of identification of 10 indigenous bacterial isolates which tested were obtained two genera of bacteria namely *Bacillus* sp and *Clostridium* sp. The identification results of 10 indigenous bacterial isolates are presented in Table 4.

**Table 4.** The identification results of 10 indigenous bacterial isolates

Isolates	Pigmentation	Colony form	Colony margin	Elevation	Identification
R	brownish yellow	irregular	undulate	raised	<i>Clostridium</i> sp
Q	white cloudy	irregular	undulate	raised	<i>Clostridium</i> sp
TLB1	white	irregular	undulate	raised	<i>Bacillus</i> sp
TLB2	brownish white	irregular	undulate	raised	<i>Clostridium</i> sp
II	white cloudy	irregular	undulate	raised	<i>Clostridium</i> sp
III	white	rhizoid	filamentous	raised	<i>Clostridium</i> sp
G	brownish white	irregular	undulate	raised	<i>Clostridium</i> sp
TLB3	white cloudy	irregular	entire	raised	<i>Clostridium</i> sp
TLB4	white cloudy	irregular	undulate	raised	<i>Clostridium</i> sp
TTC	white	irregular	entire	raised	<i>Clostridium</i> sp

The ten isolates of selected bacteria identified, nine bacterial isolates were identified as *Clostridium* sp and one bacterial isolate as *Bacillus* sp. Microbes that are tolerant of the environment contaminated with heavy metals potentially become biological agents for heavy metal accumulation. This bacterium can be used to overcome environmental pollution caused by heavy metals. Bacteria, molds, algae, and yeast can accumulate heavy metals Ag, Au, Cd, Co, Cu, Fe, Ni and Zn [16]. *Pseudomonas*, *Thiobacillus*, *Bacillus*, and N<sub>2</sub> fixing bacteria are reported to be able to accumulate heavy metals [17]. In addition, bacteria are soil microorganisms that play an important role in the process of reforming organic matter and procuring minerals for high-level plant growth processes [18]. Bacteria on the soil also play a role as producers of additives. Soil microbes are capable of producing additives such as antibiotics, biopesticides, microbial toxins, growth regulators (ZPT), and enzymes [19]. *Bacillus* is a group of bacteria that can act as phosphate solvents in the soil. *Clostridium* is a group of bacteria that can bind free nitrogen from the air. Based on the results of the above research known indigenous bacteria from the genus *Bacillus* Sp. is gram-positive (+) bacteria that do not have the catalase enzyme, do not have the ability to use carbohydrates and produce endospores in their life cycle while indigenous bacteria from the genus *Clostridium* sp. is a gram-positive (+) bacterium which in part has the enzyme catalase, all of which have the ability to use carbohydrates and only some of them produce endospores. This *Bacillus* sp and *Clostridium* are potential to be further tested so this indigenous bacteria can be used as biological agents to reduce metal toxicity on post-mining land and increase soil fertility

### 4. Conclusion

The results of this study obtained two types of indigenous bacteria from the genus *Bacillus* sp and *Clostridium* sp which have varied characterizations of biochemical tests. These bacteria are potential to be further tested so this indigenous bacteria can be used as biological agents to reduce metal toxicity on post-mining land and to increase soil fertility.

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