Avrupa Bilim ve Teknoloji Dergisi
Sayı 20, S. 206-215, Aralık 2020
© Telif hakkı EJOSAT'a aittir
Araştırma Makalesi

www.ejosat.com ISSN:2148-2683

European Journal of Science and Technology
No 20, pp. 206-215, December 2020
Copyright © 2020 EJOSAT
Research Article

Catalytic Activity of Optimized Environment and Microbial Conditions on Bioleaching of Leonardite for Bio-Humic Production

Nurgul Kıtır Şen[1*], Metin Turan[2]

[1] Gebze Teknik Üniversitesi, Yer ve Deniz Bilimleri Enstitüsü, Gebze, Kocaeli, Türkiye (ORCID: 0000-0001-7463-4186), nksen@gtu.edu.tr
[2] Yeditepe Üniversitesi, Mühendislik ve Mimarlık Fakültesi, Genetik ve Biyomühendislik Bölümü, İstanbul, Türkiye (ORCID: 0000-0002-4849-7680), m_turan25@hotmail.com

(First received 1 July 2020 and in final form 11 October 2020)

(**DOI:** 10.31590/ejosat.761458)

ATIF/REFERENCE: Kıtır Şen, N., & Turan, M. (2020). Catalytic Activity of Optimized Environment and Microbial Conditions on Bioleaching of Leonardite for Bio-Humic Production. *European Journal of Science and Technology*, (20), 206-215.

Abstract

The potential use of Leonardite for organic farming is common in worldwide but due to standart chemical production process of humic acid from Leonardite ore, the humic acid usage in organic farming is forbidden. Here we present a new optimized method for biological humic production via using a combination of biotechnological and conditional processes. This study includes two phases. Initially, the collection of Leonardite ore samples from different Leonardite deposites of Turkey and the determination the most qualified ore bed via using FTIR spectroscopy and SEM view results. Due to the results, the best ore was determined from Adana-Tufanbeyli province. In the second phase, the samples were taken from Adana-Tufanbeyli province for bio-humic production via using several bioleaching process to find the best condition for maximum production of bio-humic. California method was used as standart method to determine the leaching ratio. As an initial of bioleaching process, several leonardite ore ratios were determined in the prepared bacteria based solution then under different temperature, time conditions, the productivity effect of pH parameters were tested to find the optimum leaching standarts for bio-humic production. The optimized conditions were provided 23.08% bio-humic leaching ratio. This result shows us there is a better potential of extraction of humic acid via biotechnological methods compared with the conventional methods. Since there is a restriction of usage of humic acid in organic farming due to its chemical production process, this study opens a new way of usage of humic in biologic form in organic farming and with this high leaching potential there can be many studies can be done and new microorganism isolations can be tested with the innovative process of the study. This eligibility of this process to produce humic in biologic form provides an innovative input for organic farming as bio-humic with this study.

Keywords: Bio-humic, Organic farming, Leonardite, Microbial Consortium, Bio-leaching

Biyolojik Hümik Üretimi için Leonarditin Biyo-Liçinginde Optimize Edilmiş Ortamın ve Mikrobiyal Koşulların Katalitik Aktivitesi

Öz

The potential use of Leonardite for organic farming is common in worldwide but due to standart chemical production process of humic acid from Leonardite ore, the humic acid usage in organic farming is forbidden. Here we present a new optimized method for biological humic production via using a combination of biotechnological and conditional processes. This study includes two phases. Initially, the collection of Leonardite ore samples from different Leonardite deposites of Turkey and the determination the most qualified ore bed via using FTIR spectroscopy and SEM view results. Due to the results, the best ore was determined from Adana-Tufanbeyli province. In the second phase, the samples were taken from Adana-Tufanbeyli province for bio-humic production via using several bioleaching process to find the best condition for maximum production of bio-humic. California method was used as standart method to determine the leaching ratio. As an initial of bioleaching process, several leonardite ore ratios were determined in the prepared bacteria based solution then under different temperature, time conditions, the productivity effect of pH parameters were tested to find the optimum leaching standarts for bio-humic production. The optimized conditions were provided 23.08% bio-humic leaching ratio. This result

[*] Corresponding Author: nksen@gtu.edu.tr

shows us there is a better potential of extraction of humic acid via biotechnological methods compared with the conventional methods. Since there is a restriction of usage of humic acid in organic farming due to its chemical production process, this study opens a new way of usage of humic in biologic form in organic farming and with this high leaching potential there can be many studies can be done and new microorganism isolations can be tested with the innovative process of the study. This eligibility of this process to produce humic in biologic form provides an innovative input for organic farming as bio-humic with this study.

Anahtar Kelimeler: Biyo-hümik, Organik tarım, Leonardit, Mikrobiyal konsorsiyum, Biyo-liçing

1. Introduction

Nowadays, the developing countries determined some strategies that target to gain the maximum yield and economic products on agricultural production via using several technologies taken from developed countries. The base of these strategies were composed from increasing agricultural productivity and yield. However, the biodiversity and natural balance, sustainability of agriculture were not taken into account and unbounded chemical input usages have been caused a lot of short and long term environmental problems. To review and strengthen the soil productivity factors with dense chemical inputs are not a sustainable solution for farming. Initially, to solve the problems that causing soil degredation and soil productivity limiting factors will compose the important steps. Depending on the intensive chemical applications, increasing input usages cause addiction to use increasing chemical fertilizers, pesticides and herbicides to gain the same amount of yield due to decreasing biodiversity affects of these chemicals in the soil . Also the intensive usage of chemicals causes soil degradation. The existing part of the nature as water, air circulation, nutrient element cycle, disease and pathogens auto-control, ion change etc. cycles are affected in the soils that have lost their biodiversity and their lack cycles cause the soil productivity losses. Because of these reasons, the supporting activities should be used to increase soil biodiversity. In addition to no-till farming systems and to use the organic based productions as an input provides the organic material and biodiversity increase in 2-3 years. The obtained production will be economical and more products could be provided to the market. Humic acid and derivative organic inputs provide positive effects on soil content and problems as high pH, high lime and low organic matter etc. In Turkey there are five Leonardite sources in Bursa-Davutlar, Balıkesir-Balya, Adana-Tufanbeyli, Tekirdağ-Saray and Konya-Beyşehir. NaOH and KOH are used in chemical process for extraction of humic acid and its components but the outputs as an end of these processes can be used only in conventional farming and this chemical process is not eligible to extract valuable organic farming content as organic acid, amino acid and hormone from Leonardite sources. In the market, organic inputs and their sources are very limited and this problem is common all over the world. Organic farming is an agricultural application system and it has unique standarts. In every level of organic production, the inspection is done by the author foundation and inspectors. In order for a product to be organic, it must have an organic certificate and product label. Bursa-Davutlar, Balıkesir-Balya, Adana-Tufanbeyli, Tekirdağ-Saray and Konya-Beysehir have the most important leonardite deposits of Turkey. But the direct use of these sources are not possible. Our target is to find the best appropriate source for bio-humic production and convert that source in available forms and provide inputs for organic farming by using biotechnological process via using special PGPRs for natural extraction without chemical process. The aim of this project after pre-experiments, to convert the leonardite sources into bio-humic forms to provide inputs for cosmetic and dermacosmetic, food additive material, organic humic fertilizer input in organic farming, sustainable farming and even in conventional farming by using the identified PGPRs. These PGPRs have also demineralization capability to dissolve humic materials from the Leonardite with the enzymes they secrete.

The Humic Substances (HS) effects on plant growth have been known for so many times and HS have benefits on soil structure improvement as aeration, aggregation, permeability and water holding capacity (Tan & Nopamornbodi, 1979). In traditional farming the organic wastes and manures have been used and the important form of these organic wastes have been released as humus and to earn the humus status and other chemical and biochemical soil properties they should have heavy metal accumulation properties, organic pollutants and phytotoxic compounds. About these factors the soil and organic matters should be analysed to understand the structure and to clean from toxical structures, the organic matter structure analyses have commonly been made via FTIR "Fourier Transform Infrared Spectroscopy''(Lima et al., 2009). According to these parameters also we have made FTIR analysis on the selected leonardite samples. Humic and Fulvic acids are commonly used in organic crop production and they are a good plant growth stimulator that provides a good root, shoot and stem elongation. Due to water holding capacity of humic acid as seven times more of their volume, they provide a good nutrient taking process about their utilization of the water and it provides for plants a better yield and quality (Wright & Lenssen, 2013).

HS are end products of humification and they are complex and heterogenous mixtures of polydispersed matters as a result of conversion of plant and microbial remains via using some chemical and biochemical reactions (Muscolo, Sidari, & Nardi, 2013). The humic substances have a similar structure with hormones and acting like plant growth hormones that they increase the membrane permeability and provide the transportation of essential elements to the roots, and balance the respiration (Masciandaro, Ceccanti, Ronchi, Benedicto, & Howard, 2002).

Organic agriculture needs a systematic production method and soil is a crucial factor to provide a sustainable management on organic farming systems but soil degredation increase causes the decrease of food safety (Fließbach, Oberholzer, Gunst, & Mäder, 2007). One of the reasons of the decrease of eligible soil for farming is overdose using of chemicals. Organic farming products are limited and due to this, that type of farming is not expanded at enough level. In addition to this 96% of Turkey's farm fields have poor in terms of organic matter. The aim of the study to provide a useful new biological source for organic farming as an input via processing the natural leonardite source with biotechnological method by using several microorganisms and bioleaching. This bioleaching method can provide a potential organic extract from leonardite source. The chemical extraction method with some chemical compounds as NaOH or KOH provides only extraction of humic+fulvic acid on chemical base but can not extract organic acid, amino acid and hormone compounds from raw leonardite. Our target in the study, in addition to biotechnological extraction of humic and fulvic acid, to obtain these organic compounds next to them via using of bioleachhing properties of our isolated microorganisms for organic farming process and to determine the optimum conditions for maximum leaching of bio-humic.

2. Material and Method

2.1. Leonardit Material Selection

The five top biggest leonardite deposites was determined from Turkey as Bursa-Davutlar, Balıkesir-Balya, Adana-Tufanbeyli, Tekirdağ-Saray and Konya-Beyşehir. In each region 30 samples were taken to determine physical and chemical properties of ores via FTIR, SEM and chemical measurements. After determination of the best property providing leonardite ore is Adana-Tufanbeyli, the leonardite ore samples were used for bioleaching process.

2.2. Physical Properties Determination of Leonardite Ore

2.2.1. FTIR Spectroscopy

FTIR spectroscopy measurements were carried out in the MID IR region in the range of 600 to 4000 cm-1 using 25 IR scans for each sample. After drying the humic acids extracted from all samples, spectra were taken without any treatment. Functional group analyzes of each sample were performed by comparison of spectra. The measurements were made with samples prepared at the same pH values (Lima et al., 2009).

2.2.2 SEM Views

Scanning Electron Microscopy with a Hitachi S-3400N SEM was used to determine the samples structure is crystal or not. 5 different samples were taken from 5 different Leonardite ore bed from Turkey's different regions as Bursa-Davutlar, Balıkesir-Balya, Adana-Tufanbeyli, Tekirdag-Saray and Konya-Beysehir.

2.3. Culture Collection and Microbial Culture Preparation for Bioleaching Process (Media Composing Process for Bioleaching)

Pseudomonas putida (ATCC® 47054D-5), *Zygosaccharomyces bailii* (ATCC® 8766) *and Rhodotorula mucilaginosa* (ATCC® 2503), *Saccoramices cerevisiae*(ATCC® 18824),, *Bacillus subtilis* (ATCC® 21332), *Bacillus megaterium* (ATCC® 25848), *Aspergillus niger* (ATCC® 20057), and *Thiobacillus ferrooxidans* (ATCC® 8158), isolates for bioleaching were obtained from Yeditepe University Microbiology Laboratories. For media, agar and vinasse is used. A consortium was composed at 10^{12} cfu/ml density.

2.4. The Process of Leonardite Ores Standart Humic Process

The leonardite ore was milled on 200–400 mesh "37–74 mm'' dimensions. The milled leonardite ore''400 gr.'', water''575 gr.'' and NaOH ''25 gr.'' was put in reactor. The reactor temperature was 100 °C with obtained steam from steam generator. When the temperature was under or upon 100 ºC, the control panel gives direction to steam generator with the taken infos from thermocouples. The reactor was worked for 90 turnover/min and 24 hours. When the work was over, the reactor was cooled and the pressure was pumped out. The media was passed from centrifuge decanter for separation of liquid and solid form. Some of humic matters and non humic matters in leonardite ore were solved in water. The non soluble part of leonardite ore stayed as solid phase and was seperated via decanter from liquid phase.

2.5. Bioleaching Methods

Different Leonardite/media ratios "20, 30, 40, 50, 60, 70%''were selected. The media pHs were selected as 6.5, 7.0, 7.5, 8.0, 8.5. Different days were selected as "1, 2, 3, 4, 5, 6 and 7''.Different temperatures as "10, 20, 30, 45, 50 °C'' were selected for optimization of bioleaching process. The temperature and pH were controlled during the fermentation. Each day the samples were taken and laboratory analysis and controls were made in aceptic conditions.

2.6. TSE and California Methods

For Leonardite as its solid material for humic acid measurement we used TSE Method. For Liquid humic acid ratio from biohumic and standart humic material we used California method.

2.6.1 TSE 5869 ISO 5073 Black coal and Lignites

0.2 gr solid form Leonardite ore was taken for the humic acid extraction. 150 ml alkaline Na4O7P2(Sodium pyrophosphate) was added and the leonardite ore was put in boiling water. After 2 hours boiling, the material was cooled to room conditions and diluted to 200 ml in erlenmayer flask. Then washing and filtering process was done. 5 ml material was taken and 5 ml $K_2Cr_2O_7$ solution and 15 ml H2SO4 solution were added and put in hot water bath for 30 min. After cooling dilution to 100 ml, 3 ml phenon-troline indicator was added. Titration was done with (NH4)2Fe(SO4)2 until colour to tile red.

2.6.2. CDFA Method

After bioleaching, the obtained liquid form was used to measure Humic+Fulvic acid contents via using the gravimetric Humic Acid - California Department of Food and Agriculture (CDFA) (1999) method.

2.7. Statistical Analysis

The statistical analysis were made of the obtained results from study with Minitab 19 program.

3. Results and Discussion

3.1. The Initial Analysis Results of leonardite Samples

The FTIR spectroscopy measurements have been made via using IR Prestige-21 in MID IR region and between 600 to 4000 cm-1 and 25 scanning for each sample. The extracted humic acids spectrum were taken after drying including no process from all samples "Table 1, Figure 1".The comparison of spectrum with functional group analysis belong to each samples have been made. Generally, wide and overlapping pics revealed between 3600-2000 cm-1 region. The wide band between 3400–3000 cm-1 range showed hydroxyl groups that bonded with hydrogen bonds. C-H distention vibrations that should be seen in 3100-3000 cm-1 region, lost in wide bonds. The peaks in 2920–2850 cm-1 region showed distention vibration belong to aliphatic CH, CH2, CH3 groups related with aromatic groups. OH vibration is in shoulder carboxyl groups of 2570 cm-1 region. The mutual seen bonds in spectrums, 1712 cm-1 bond show the peak belong to carboxyl, aldehite and ketone carbonyl but according to state of carboxyl group the height of the peak can be changed by H bonded state or ionizated state and shows differences based on peak heights in humic acids from isolated different regions. This difference was sourced from the vibration differences of COOH groups acid and salt forms. In the state of conversion COOH groups into COO-, in the height of 1380 cm-1 peak, the increases occur compared to 1712 cm-1 peak. This state showed differences between carboxyl group amounts from the samples obtained from different regions. Because same steps and same chemicals have been used for determination of humic acids. The peak in 1620 cm-1 occurs from carbonyl groups quinons and nitrates belong to amide groups. The peak at 1600 cm-1 releases from C=C group took place in aromatic structure and hidrogen bonded C=O group. Approximately in the 1540 cm-1 bond was amide bonds from peptide groups and the peak in 1510 cm-1 was aromatic C=C group. It is thought that 1460 cm-1 peak was sourced by aliphatic C-H slope vibration. The peak group between 1290–1220 cm-1 was sourced by C-O distention and OH degradation. The peak in 1120 cm-1 is released from alyphatic CH2, OH or C–O groups and the peak group at 995 cm-1 was released from C-O regression vibration and Si-O occurs belong to silicates "Table 1".

Table 1. UV Spectra Results "N=30'' Taken From 5 Different Points of 30 Samples Average

Sample	365 nm	465 nm	665 nm	E4/E6 Ratio
Adana-Tufanbeyli	0.452	0.277	0.215	1.29
Balıkesir-Balya	0.625	0.284	0.185	1.54
Bursa-Davutlar	0.336	0.196	0.199	0.98
Konya-Beyşehir	0.233	0.146	0.143	1.02
Tekirdağ-Saray	0.372	0.232	0.194	1.20

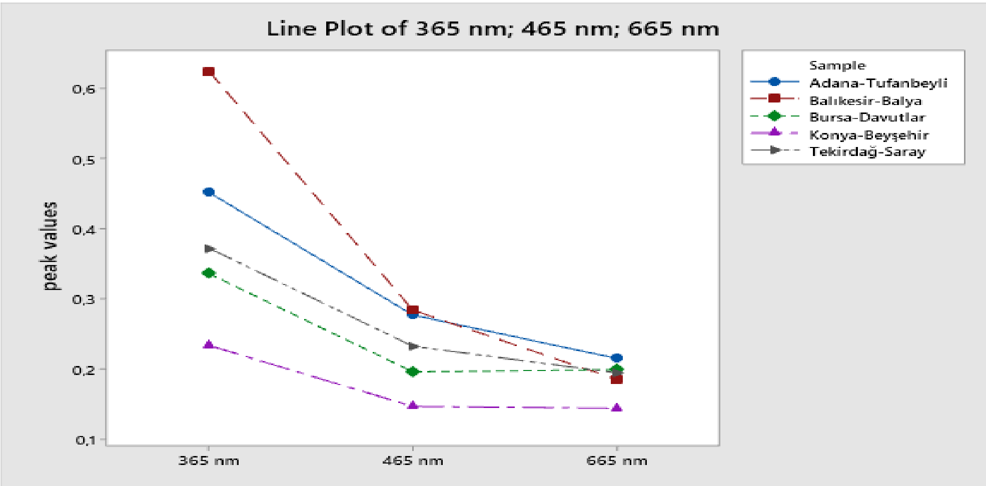

Fig. 1. FTIR Spectroscopy Measurements of Samples From 5 Different Locations

SEM was made to see the samples structure if christal or not. The SEM analysis results from 5 different location and due to carrying of the properties of most humic including structure, Adana Tufanbeyli sample showed better quality for humic production "Figure 2". According to the SEM views the samples were determined at non chrystal structure but found in polymeric or macro molecular structure.

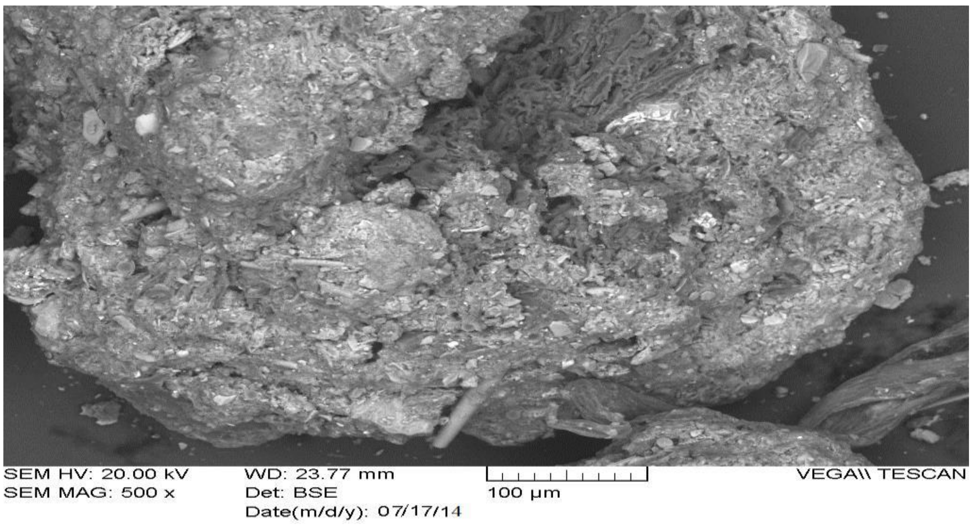

Fig. 2. SEM View of The Sample from Adana-Tufanbeyli Location (Humic Acid Ratio Of Leonardite is 59.5 %).

The measurements were made with the same pH value prepared samples. A similarity were determined in FTIR spectrums of different regions samples "Figure 3".

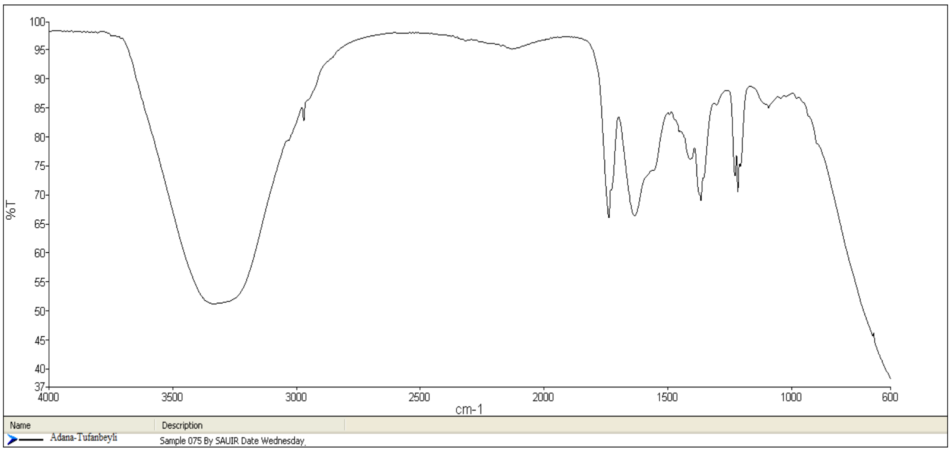

Fig. 3. FTIR Spectra from Adana-Tufanbeyli

When the obtained spectrums were compared, the aliphatic group amounts in structure showed differences. In this situation, the highest probability, in some samples the humic material composing could't be defined totally. In addition to this a significant difference occured in the hight of 995 cm-1 peak. There was a big possibility to a big range in addition to carbonhydrates, clay silica and minerals. In "Table 1" the humic acid FTIR and FTIR spectrum possible vibrations and belonging groups were shown "Table 2-6".

Table 2. 30 Samples Averages in FTIR Spectra Bond Vibration Groups From 5 Different Locations.

cm-1	**Groups**
3626	N-H
3410-3083	H-bonded O-H
2957-2849	Aliphatic C-H
1739	C=O ''esther''
1720-1705	C=O ''ketone ve/veya carboxylic acid''
1700	C=O ''amine''
1665	C=C, C=N ''aliphatic imine''
1661	C=C, aromatic C-C
1659-1537	C=C
1537-1514	N-H ''amide''
1511	COO-
1506	Aromatic C=C
1455	N-H ''amine ve imine''
1462-1450	Aliphatic C-H
1420-1332	O-H ''alcohol ve phenol''
1330-1315	O-H ''alcohol ve phenol'' ve C-H ''alkene''
1270-1263	O-H ''alcohol'' ve C-O-C ''arilether''
1228	O-H ''alcohol ve phenol'' ve C-O-C''arilether''
1224-1199	O-H ''phenol'' ve C-O-C ''arilether''
1161-1146	C-O ''alcohol'', C-O-C ''ether''
1126-1124	Aromatic C-H, C-O-C ''ether''
1116	C-O-C ''ether'', C-N ''amide''
1107-1083	C-O ''alcohol'', C-O-C ''ether''
834-795	C-H ''alkine''

Table 3. Chemical Analysis Results of 30 Samples Average Taken From Different 5 Locations “N=30’‘

Region	**Humidity %**	**Ash%**	**S%**	**F.M.%**	**H.A.%**	**O.M.%**	**C %**	**pH**	**N %**	**E.C dS/cm**	**CEC cmol/kg**
Adana / Tufanbeyli	41	28	2.1	24	59.5	63.1	36.5	4.8	3.25	1210	205
Konya / Beyşehir	48	25	1.1	24	42.4	52.4	30.39	4.2	2.32	1050	184
Bursa / Keles	31	26	4.5		27.28	39.42	22.86	4.6	1.67	1037	160
Tekirdag / Saray	45	16	1.9	20	24.45	30.6	17.74	5	1.43	985	134
Balıkesir / Balya	5	55		22	22.04	36.08	21.34	4.7	1.64	968	152

Table 4. The Chemical Analysis Results of Obtained Product From Ph 8.0, 45°C Incubation, 144 H Duration, 60% Ore And 40% Microbial Solution Ratio

Properties	**Values**
pH	8.0±0.35
EC ‘‘mS/m’‘	55.80±1.75
Organic Matter ‘‘%’‘	22.24±0.10
Total Nitrogen	0.65±0.05
Total Phosphorus ‘‘P2O5%’‘	1.14±0.06
Total Potassium ‘‘K2O%’‘	5.74±0.23
Total Calcium ‘‘%’‘	0.54±0.02
Total Magnesium ‘‘%’‘	0.26±0.03
Total Sulphur ‘‘%’‘	0.75±0.02
Total Cu ‘‘ppm’‘	0.35±0.03
Total Fe ‘‘ppm’‘	2375.13±10.75
Total Mn ‘‘ppm’‘	5.66±0.30
Total Zn ‘‘ppm’‘	4.85±0.04
Total Ni ‘‘ppm’‘	3.34±0.82
Total Cd ‘‘ppm’‘	0.18±0.02
Total Cr ‘‘ppm’‘	0.25±0.07
Total Co ‘‘ppm’‘	0.14±0.03
Total Pb ‘‘ppm’‘	0.10±0.01
F ‘‘ppm’‘	0.13±0.01
Si’‘ppm’‘	5.14±0.01
Al’‘ppm’‘	2.83±0.01

Table 5. The Hormone Analysis Results of Obtained Product From Ph 8.0, 45°C Incubation, 144 H Duration, 60% Ore And 40% Microbial Solution Ratio

Hormone	**’‘ng/ul’‘**
Giberallic acid	13450±12.5
Salicylic acid	11523±14.7
IAA	466±2.3
ABA	1.85±0.24

Table 6. The Amino Acid Analysis Results of Obtained Product From Ph 8.0, 45°C Incubation, 144 H Duration, 60% Ore And 40% Microbial Solution Ratio

Amino acid content	Pmol/µl
Aspartate	44.54±0.82
Glutamate	3.60±0.66
Asparagine	23.65±0.9
Serine	48.74±1.2
Glutamine	5.64±0.1
Histidine	67.12±5.6
Glycine	46.78±0.9
Theonine	29.35±0.7
Arginine	45.36±1.4
Alanine	3.42±0.1
Tyrocine	38.55±1.3

As a result of settling molecules, the composed humic material powder includes nano dimensioned structures. This result also covers the recommended structure for humic material and chemical analysis were made for 5 different locations and for each location 30 samples were taken and the average of these samples were identified including humidity%, ash%, S%, Fulvic Matter%, Humic Acid% and Organic Matter%, C%, pH, N%, EC dS/cm, CEC cmol /kg "Table 3".

After all these measurements due to its humic acid structure, and the best organic matter and humic acid ratio and the other contents, Adana-Tufanbeyli Leonardite ore bed was identified for best qualified ore between the other ores (Konya-Beysehir, Bursa-Keles, Tekirdag-Saray, Balıkesir-Balya).

3.2. Statistical analysis

The statistical analysis was made with the help of Minitab 19 program. Firstly, contour plot of bio-humic production ratio dur to temperature and pH conditions and the maximum efficiency of bio-humic production was between at 7 to 9 pH and temperature at 40- 50 °C "Figure 4". The response optimization of temperature and Ph for bio-humic production analysis were made with the obtained results. The optimum temperature was determined as 47.17 and optimum pH at this level was 8.0 "Figure 5". The surface plot of temperature and pH for bio-humic production were made at this levels the leaching amount of bio-humic was close to 16% "Figure 6". Main effects plot was made for bio-humic production and between 40 to 50 °C temperature, the leaching ratio was reached the maximum amount as between 15 to 16% and at Ph 8, the leaching ratio reached 15% "Figure 7". The interaction plot was made also for bio-humic production and ph at 10 showed the lowest efficiency for bio-humic leaching betweeen 7-9%. The pH at 6.5 value showed an increase when compared pH at 10. The fitted means of interaction plot for bio-humic production, 8.25 pH was provided the best efficiency for bio-humic leaching between 12-16% "Figure 8". For the optimization after the contour plot and response optimization results the range for ph were taken between 7 to 9 and temperature range was taken 45 and 50 °C, time range was selected from 1 to 7 days and leonardite ore ratio was taken from 20 to 70%.

According to response optimization results of these parameters, pH at 8.0, temparature at 45 ºC, 6.5 days and 60% leonardite ore usage conditions were determined the best for bio-humic production. The optimized conditions were provided 23.08% bio-humic leaching ratio "Figure 9".

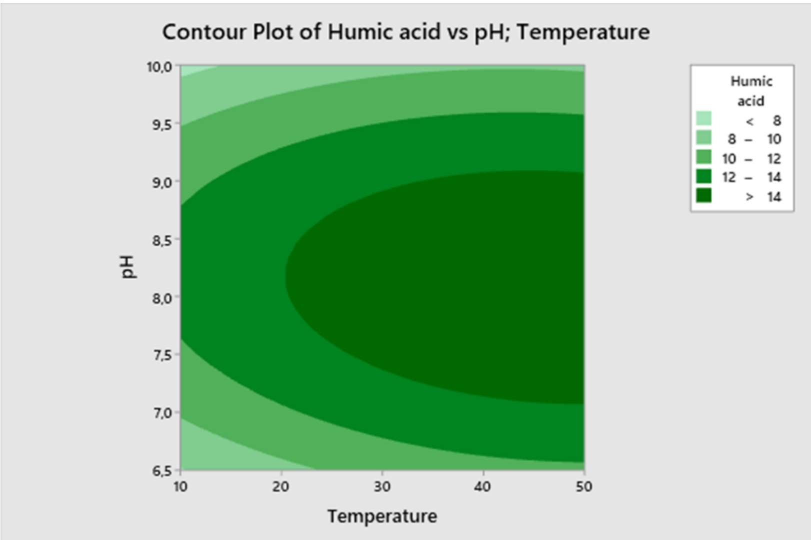

Fig. 4. Contour Plot of Bio-Humic Acid Production Ratio due to Temperature and pH Conditions

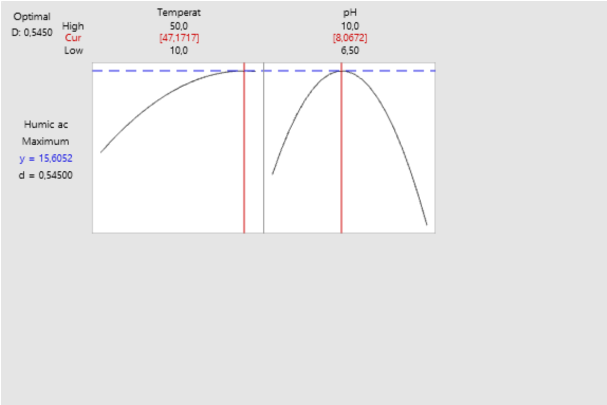

Fig. 5. Response Optimization of Temperature and pH for Bio-Humic Acid Production

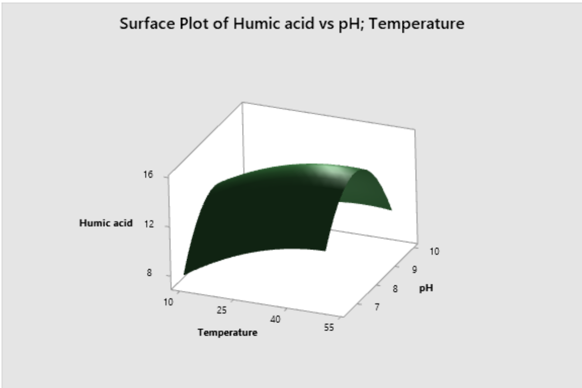

Fig. 6. Surface Plot of Temperature and pH for Bio-Humic Acid Production

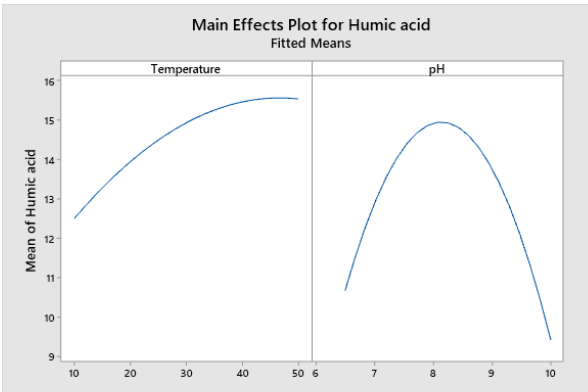

Fig. 7. Main Effects Plot of Temperature and pH for Bio-Humic Acid Production

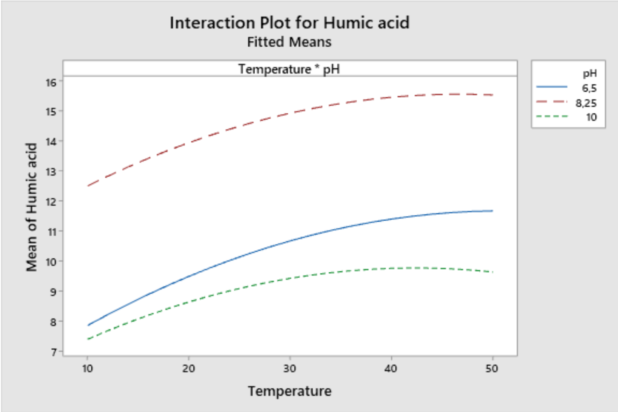

Fig. 8. Interaction Plot of pH and Temperature for Bio-Humic Acid Production

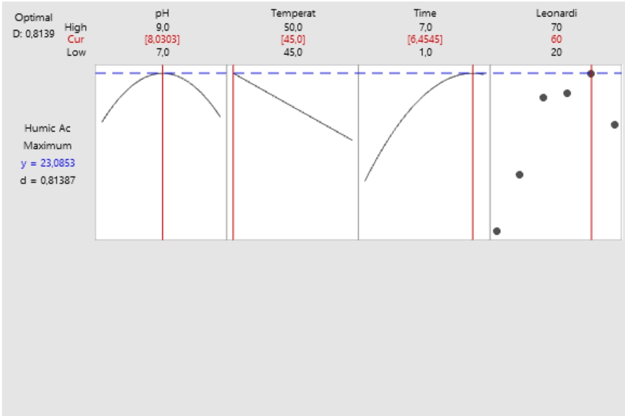

Fig. 9. Response Optimization of Time, Leonardite Ore Ratio with Eliminated Temperature and pH for Bio-Humic Acid Production

3.3. Biologic Humic Acid Production Method Optimization Results

For determined to optimization diffrent pH, temperature and ore/microbial solution ratio on tests for biologic humic acid obtaining from leonardite sources, some specific microorganisms as *Pseudomonas putida* (ATCC® 47054D-5), *Zygosaccharomyces bailii* (ATCC® 8766) *and Rhodotorula mucilaginosa* (ATCC® 2503), *Saccoramices cerevisiae*(ATCC® 18824),, *Bacillus subtilis* (ATCC® 21332), *Bacillus megaterium* (ATCC® 25848), *Aspergillus niger* (ATCC® 20057), and *Thiobacillus ferrooxidans* (ATCC® 8158), consortium was used to extract the leonardite ore with help of environmental condition optimization method. Individual and group applications were made for obtained biologic humic acid compounds and the efficiency degrees were detemined of humic + fulvic acids via optimization tests of California method. The most effective product from pH 8.0, 45°C incubation, 6-7 days duration, 60% ore and 40% microbial solution ratio, and some chemical "Table 4", hormone "Table 5", amino acid "Table 6", organic acid "Table 7" content was the much more high value compare the standart methods and different pH, ratio and temperature degree. As seen from the figures and tables, we have finished the extraction of humic and fulvic acids from leonardite ores in selected pH, temperature, day and ore/solution ratios "Table 4".

Table 7. The Organic Acid Analysis Results of Obtained Product From Ph 8.0, 45°C Incubation, 144 H Duration, 60% Ore And 40% Microbial Solution Ratio

Organic acid	**"ng/ul"**
Oxalic acid	6.75±0.2
Propionic acid	6.89±0.1
Tartaric acid	2.14±0.05
Butyric acid	2.78±0.4
Malonic acid	5.12±±0.3
Malic acid	6.33±0.03
Lactic acid	8.56±0.7
Citric acid	7.21±0.5
Maleic acid	1.66±0.2
Fumaric acid	1.85±0.1
Succinic acid	9.95±0.9

4. Conclusions and Recommendations

A new optimized method was produced in the study for biologic humic acid production via using a combination of biotechnological and environmental conditions optimization. This study provided a new source for organic farming as biological humic acid via using the several enzymatic activities of different microorganism consortium. Humic acid is a very good soil regulator and input for farming but until this time it was not possible to use in organic farming due to its chemical production process. The study provides also humic acid usage possibility for organic farming due to its fitted biotechnological process. There are many specific leaching bacterial population in all over the world. This biological humic acid production method will provide to test the maximum efficiency of other bacterium chains and consortiums. Due to its organic and rich content of biologic humic acid, not only humic acid was leached but also several beneficial hormones were absorbed from leonardite ores can increase the efficiency of preparation. In the further studies this preparation efficiency will be tested on many kinds of plants. And several preparation of biologic humic acid efficiency can be tested on many plants growing period and productivity after applying this method with different consortium of bacteria.

4. Acknowledge

This study was entirely funded by TUBITAK with SANTEZ O9032015 coded project. The study is created from Ph.D. thesis data of Nurgül Kıtır in Yeditepe University and laboratories.

References

Fließbach, A., Oberholzer, H.-R., Gunst, L., & Mäder, P. (2007). Soil organic matter and biological soil quality indicators after 21 years of organic and conventional farming. Agriculture, Ecosystems & Environment, 118(1-4), 273-284.

Lima, D. L., Santos, S. M., Scherer, H. W., Schneider, R. J., Duarte, A. C., Santos, E. B., & Esteves, V. I. (2009). Effects of organic and inorganic amendments on soil organic matter properties. Geoderma, 150(1-2), 38-45.

Masciandaro, G., Ceccanti, B., Ronchi, V., Benedicto, S., & Howard, L. (2002). Humic substances to reduce salt effect on plant germination and growth. Communications in soil science and plant analysis, 33(3-4), 365-378.

Muscolo, A., Sidari, M., & Nardi, S. (2013). Humic substance: relationship between structure and activity. Deeper information suggests univocal findings. Journal of Geochemical Exploration, 129, 57-63.

Tan, K., & Nopamornbodi, V. (1979). Effect of different levels of humic acids on nutrient content and growth of corn (Zea mays L.). Plant and soil, 51(2), 283-287.

Wright, D., & Lenssen, A. W. (2013). Humic and fulvic acids and their potential in crop production.