Agrotechnology Research Journal
Volume 7, No. 2, December 2023, pp. 126–131
ISSN 2655-7924 (Print)
ISSN 2614-7416 (Online)
https://jurnal.uns.ac.id/arj
doi:10.20961/agrotechresj.v7i2.79946

Iron Contaminated Soils Remediation Using Secondary Metabolites of *Trichoderma harzianum* T10 and Its Effect on Spinach Growth

***Loekas Soesanto*[1]*, *Murti Wisnu Ragil Sastyawan*[2], *Endang Mugiastuti*[1], *Tamad Tamad*[1]**

[1]*Faculty of Agriculture, Universitas Jenderal Soedirman, Purwokerto, Central Java 53122, Indonesia*
[2]*Faculty of Technique, Universitas Jenderal Soedirman, Purbalingga, Central Java 53371, Indonesia*

Received **26 October 2023**; Accepted **29 November 2023**

ABSTRACT
Iron (Fe) is an essential metal whose presence in excess can pollute the environment, cause toxic effects on plants, and degrade soil quality. Efforts have been made to overcome this by remediation using secondary metabolites of *Trichoderma harzianum* T10. This study aimed to determine the potency and appropriate concentration of *T. harzianum* T10 secondary metabolites as a remediator for Fe-contaminated soil and its effect on the growth and yield of spinach grown on remediated soil. The research was conducted at the Screen House, Soil Laboratory, and Plant Protection Laboratory, Faculty of Agriculture, Universitas Jenderal Soedirman for four months. A randomized block design was used with treatment consisting of control and secondary metabolites application of *T. harzianum* T10 concentrations of 25, 50, 75, and 100%, repeated five times. The secondary metabolites were applied in the afternoon by pouring it on the soil in polybags and letting it stand for 10 days in tightly closed conditions. The variables observed were Fe content in the soil, plant height, number of leaves, shoot fresh weight, fresh root weight, and root length of spinach plants. The results showed that the secondary metabolites of *T. harzianum* T10 have the potential to remediate iron-congested soil. The content of Fe in the soil is 823 ppm. The appropriate concentration of *T. harzianum* T10 secondary metabolites as a remediator for Fe-contaminated soil is 50%, which can reduce the content of Fe (iron) by 46% compared to controls. The application of *T. harzianum* T10 secondary metabolites has not affected the growth and yield of spinach, which was grown on remediated soil, although there is a tendency to be better.

Keywords: Metal pollution; Microbial antagonist; Organic remediation; Soils
***Cite this as (CSE Style)*:** Soesanto L, Sastyawan MWR, Mugiastuti E, Tamad T. 2023. Iron contaminated soils remediation using secondary metabolites of *Trichoderma harzianum* T10 and its effect on spinach growth. Agrotechnology Res J. 7(2):126–131. https://dx.doi.org/10.20961/agrotechresj.v7i2.79946.

PENDAHULUAN

The content of heavy metals in soil is naturally very low unless the soil is polluted (Chibuike and Obiora 2014). Metals can enter the soil through different sources, becoming pollutants. Fertilizers, pesticides, added organic and inorganic materials, waste residues, and activated sludge contain some heavy metals (Alengebawy et al. 2021). Fe (iron) metal is an essential metal whose presence in excessive amounts can have a toxic effect (Abbaspour et al. 2014). Excess Fe deposits in the soil can reduce soil quality (Strezov and Chaudhary 2017). High levels of Fe metal also affect humans, such as poisoning (vomiting), intestinal damage, premature aging to sudden death, arthritis, bleeding gums, cancer, cirrhosis, constipation, diabetes, diarrhea, dizziness, fatigue, hepatitis, high blood pressure, and insomnia (Myers [date unknown]).

Efforts can be made to reduce Fe content in the soil, which can cause these negative impacts, including by chemical means (Lindsay and Schwab 1982), salinity (Shrivastava and Kumar 2015), phytoremediation (Yan et al. 2020), and bioremediation (Kapahi and Sachdeva 2019). Remediation is the development of environmentally friendly biotechnology by utilizing various processes to control pollution (Kensa 2011). Bioremediation is a technology that removes or converts hazardous pollutants, such as heavy metals, into less hazardous substances and/or removes toxic elements from polluted environments, or decomposition of organic matter and final mineralization of organic matter into carbon dioxide, water, nitrogen gas, and others using dead or living biomass (Kapahi and Sachdeva 2019). De Oliveira and Tibbett (2018) mentioned that bioremediation of soil contaminated with heavy metals Cd and Zn uses ectomycorrhiza. In general, remediation is carried out using living materials, both macro- and microbes, while the use of secondary metabolites compounds from microbes has never been done.

**Corresponding Author*:
E-Mail: lukassusanto26@gmail.com

The results of Li et al. (2015) research stated that the liquid of Trichoderma spp. was able to absorb Fe metal of 30 %. *Trichoderma harzianum (T. harzianum)* can produce bioactive metabolites, such as chitinase and β-1,3 glucanase enzymes, antibiotics, toxins, and growth hormones (Vinale et al. 2009). Secondary metabolite compounds of *T. harzianum* T10 have never been used to remediate Fe metal in soil and test their effectiveness in plants. So far, the secondary metabolites of *T. harzianum* T10 have been applied to overcome plant disease problems (Soesanto et al. 2020; Soesanto et al. 2022). This study aims to determine the appropriate concentration of *T. harzianum* T10 secondary metabolites as a remediator of Fe-contaminated soil and its effect on the growth and yield of spinach plants grown in remediated Fe-contaminated soil.

BAHAN DAN METODE

The research was conducted in a screen house with preparation in the Soil Laboratory and Plant Protection Laboratory, Faculty of Agriculture, Universitas Jenderal Soedirman for 4 months.

Preparation of *T. harzianum* T10 isolate

T. harzianum T10 isolate isolated from ginger root (Soesanto et al. 2005), was maintained on PDA and incubated for 5 days at room temperature.

Production of secondary metabolites of *T. harzianum* T10

Secondary metabolites of *T. hazrainum* T10 were produced by using a PDB medium, by inserting 2 cork drill colonies of *T. harzianum* T10 into a 250 mL Erlenmeyer containing 100 mL PDB. Then, it was shaken using an orbital shaker (Daiki) at a speed of 150 rpm for 7 days at room temperature. After shaking was complete, the conidia density was calculated using a hemocytometer until a density of 1×10^7 conidia mL^{-1} was obtained. The solution was then centrifuged to separate the fungal propagules at 3000 rpm for 9 minutes and continued with filtration using Whatman filter no. 1. The supernatant formed from a density of 1×10^7 conidia mL^{-1} was used for further research as a 100% concentration.

Preparation of Fe-contaminated soil

The ultisol soil used had a cation exchange capacity of 13%, moisture content of 33.17%, field capacity moisture content of 50%, and $FeSO_4$ of 34.19 ppm. $FeSO_4.7H_2O$ was applied to reach a minimum content of 200 ppm (control) as a criterion for Fe contamination, as much as 1.744 g for 1.4 kg of soil, which was dissolved in 170 mL of water. The solution was poured evenly on the planting media and then incubated for 2 hours for ionization, namely the change of $FeSO_4$ into Fe ions (Zhou et al. 2018). Ready-to-use soil was placed in polybags as much as 3 kg $polybag^{-1}$.

Secondary metabolites application

Secondary metabolites of *T. harzianum* T10 were applied every five days in a 10-day remediation period. The concentrations used in this test were 0 (control), 25, 50, 75, and 100% by adding water. The application was done in the afternoon by pouring into the soil polybags and allowed to stand for 10 days under tightly closed conditions. This study was designed with a group randomized design with 5 treatments and 5 replications, so there were 25 experimental plot units and each experimental unit contained 3 spinach plants.

Spinach plant preparation

The spinach seeds used were of the Maestro variety. The seeds were sown first in a medium of soil mixed with sand (1:1), and moistened with water if needed. Spinach seedlings that grow with two leaves are ready for use.

Planting of spinach plants

Ultisol soil after incubation was mixed homogeneously with 37.5 g of mature cow manure per polybag. Next, three planting holes were made in each polybag, and spinach seedlings were planted. Maintenance was carried out by watering if necessary and manual weed management.

Variables observed

The observed variables included Fe content in the soil (ppm), plant height (cm), number of leaves (strands), root fresh weight (g), crown fresh weight (g), and root length (cm). Fe content in the soil was observed using the method according to Ferreira et al. (2018) and Dusengemungu et al. (2022) with an atomic absorption spectrophotometer (SSA/AAS) brand UV-VIS (V-1100 D) at a wavelength of 515 nm.

Data analysis

Data were analyzed with the F test. If significantly different, followed by DMRT (Duncan's Multiple Range Test) at a 5% error level.

HASIL DAN PEMBAHASAN

The results of Fe content analysis in the soil after $FeSO_4$ application are shown in **Table 1**.

Table 1. Fe content in the soil before and after FeSO4 application

Variable	Before	After	Percentage gain
Fe content in the soil	34.19 ppm	823 ppm	96%

It appears that the Fe content in the soil has increased and has contaminated the soil. Adding $FeSO_4.7H_2O$ to the soil can increase the iron content to 96%. This means that iron in the soil has reached a critical stage for plant growth and soil ecology. Fe content in the soil has increased and has contaminated the soil after adding $FeSO_4.7H_2O$ at 823 ppm (Table 1). This is in accordance with the lowest limit of Fe content in the soil according to WHO/FAO, which is 300 mg kg-1 or 300 ppm (Chiroma et al. 2014). The contamination of soil by Fe can have an impact on plants grown on the soil or land (Zahra et al. 2021). Crops planted on the land must first be tested for Fe levels. Fe in the soil can reduce soil productivity and overall soil yield. This is in accordance with the opinion of Rout and Sahoo (2015) also with Gambus and Wieczorek (2012), which states that the accumulation of metals in the soil because of the application of chemical fertilizers can result in decreased microbial activity, fertility, soil quality, and the entry of toxic materials into the food chain. Furthermore, Zahra et al. (2021) stated that Fe poisoning causes severe morphological and physiological disorders in plants, including a decrease in germination percentage, disrupting enzyme activity, nutrient imbalance, membrane damage, and chloroplast ultrastructure. In addition, it also causes severe toxicity to important biomolecules, leading to ferroptosis cell death and impacting structural changes in the photosynthetic apparatus, resulting in delayed carbon metabolism.

The application of secondary metabolites of *T. harzianum* T10 had a very significant effect on the effectiveness of Fe absorption, which led to a decrease in Fe content in the soil and can be seen in Table 2. The best percentage reduction in Fe content in the soil was found in the application of secondary metabolites *T. harzianum* T10 50% by 46% when compared to the control and higher Fe reduction than other treatments. Although the doses of secondary metabolites of *T. harzianum* T10 were not significantly different and all were able to reduce Fe content in the soil, the 50% dose of secondary metabolites of *T. harzianum* T10 was the highest in reducing Fe content in the soil (Table 2). The application of secondary metabolites of *T. harzianum* T10 50% in the soil is the right concentration in remediating soil contaminated with Fe. Fe remediation using secondary metabolites of *T. harzianum* T10 is a new type of remediation. In general, remediation of heavy metal-contaminated soil uses plant material (phytoremediation) (Wang and Chao 2020) or microbes (bioremediation) (Mishra 2017). Biosorption, which depends on the specialized structure of the cell wall, was found to be the main mechanism (Jin et al. 2018). Biosorption is the removal of metals from a solution using biological materials. Heavy metal removal occurs through passive binding to non-living biomass from a solution (Hansda et al. 2015; Redha 2020). Remediation has been carried out using plant secondary metabolites compounds. Plants use different strategies and a complex array of enzyme and non-enzyme anti-oxidative defense systems to overcome the overproduction of ROS caused by heavy metals entering their cells through the leaf and/or root systems (Lajayer et al. 2017). Accordingly, microbial secondary metabolites, in this case from *T. harzianum* T10, also overcome Fe contamination using enzyme and non-enzyme oxidative compounds. This is thought to be because the application of secondary metabolites of *T. harzianum* T10 has not been able to increase the number of leaves, in accordance with the statement of Herliana et al. (2018) that the application of secondary metabolites of *T. harzianum* T10 has not been able to increase the number of leaves and leaf width. This is because the content of hormone compounds in the secondary metabolites of *T. harzianum* T10 is very small so the effect is not yet visible.

Fe concentration in the soil can decrease in addition to being caused by the remediation of secondary metabolites of *T. harzianum* T10. Fe can also be absorbed by plants. According to Zahra et al. (2021), iron toxicity is characterized by the appearance of symptoms of ferric iron (Fe3+) deposits that settle on the outer layer of the roots. As a result, plant roots become few, coarse, and short so that the fresh weight of the roots decreases. Increased iron concentration in the nutrient solution medium can inhibit root elongation (Guo et al. 2022). The presence of Fe deposits can also inhibit the absorption of nutrients, such as P, which causes deficiencies in the crown of the plant so that the fresh weight of the crown decreases (Mimmo et al. 2014).

Effect of remediator on spinach.

Based on the analysis results (Table 3), the application of different secondary metabolites of T. harzianum T10 did not show significant differences in all spinach growth variables. It is suspected that the Fe content in the soil is still relatively low so the effect of T. harzianum T10 secondary metabolites application has not been seen. However, spinach plants grown in soil applied with secondary metabolites *T. harzianum* T10 tended to have higher growth than the control, although the difference was not significant.

Plant growth variables showed no significant differences between treatments (Table 3). In the control, the plant height was normal, there were no symptoms of morphological damage to the plant stem and it was suspected that the presence of Fe only inhibited spinach growth, and did not cause poisoning in spinach plants. Other variables, such as the number of leaves, crown fresh weight, root fresh weight, and root length in each treatment either with the addition of secondary metabolites of *T. harzianum* T10 or control showed results that were not significantly different (Table 3). This condition is in line with the plant height data. The secondary metabolites of T. harzianum T10 can affect spinach growth. The application of secondary metabolites of Trichoderma sp. can be utilized to help plant growth in environments containing heavy metals.

Table 2. Effectiveness of secondary metabolites of *T. harzianum* T10 as the remediator of iron content in the soil

Soil application with secondary metabolites of *T. harzianum* T10 (%)	Fe content in the soil (ppm)	Remediation effectiveness (%)
No treatment	823	0
25	759	8
50	448	46
75	682	17
100	659	20

Table 3. Growth of spinach planted in the soil remediated by a secondary metabolite of *T. harzianum* T10

Soil application with secondary metabolites of *T. harzianum* T10 (%)	Crop Height (cm)	Number of Leaves	Crown Fresh Weight (g)	Root Fresh Weight (g)	Root Length (cm)
No Treatment	20.45	2.68	1.78	0.96	2.89
25	2.95	2.80	1.91	0.97	3.08
50	22.75	2.92	2.00	0.99	2.93
75	20.85	2.72	1.71	0.95	2.95
100	20.85	2.82	1.81	0.97	2.90

Note: Numbers followed by the same letter in the same column indicate the effect is not significantly different based on DMRT at 5% error level

According to Zahra et al. (2021), excess Fe accumulated causes a decrease in plant height. Shiwachi et al. (2006) also argued that yam (*Dioscorea* spp.) plants treated with 60 mg L-1 Fe can reduce plant height by 65%. The application of secondary metabolites *T. harzianum* T10 at 50% concentration tended to be better than other treatments. This condition is thought to be the result of the decrease in Fe contamination in the soil and the result of the influence of growth hormones contained in secondary metabolites. It is known that bioactive compounds produced by *T. harzianum* include growth regulators (Cai et al. 2013; Al-Askar 2016).

CONCLUSIONS

The right concentration of *T. harzianum* T10 secondary metabolite as a remediator of Fe-contaminated soil is 50%, which can reduce the Fe content in the soil by 46%. The application of secondary metabolites of *T. harzianum* T10 does not affect the growth and yield of spinach plants grown in remediated soil.

REFERENCES

Abbaspour N, Hurrell R, Kelishadi R. 2014. Review on iron and its importance for human health. Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences. 19(2):164–74.

Al-Askar AA. 2016. Bioactive compounds produced by *Trichoderma harzianum* 1-SSR for controlling *Fusarium verticillioides* (Sacc.) nirenberg and growth promotion of *Sorghum vulgare*. Egyptian Journal of Biological Pest Control. 26(2):379–386.

Alengebawy A, Abdelkhalek ST, Qureshi SR, Wang M-Q. 2021. Heavy metals and pesticides toxicity in agricultural soil and plants: Ecological risks and human health implications. Toxics. 9(3):42. https://doi.org/10.3390/toxics9030042.

Cai F, Yu G, Wang P, Wei Z, Fu L, Shen Q, Chen W. 2013. Harzianolide, a novel plant growth regulator and systemic resistance elicitor from *Trichoderma harzianum*. Plant Physiology and Biochemistry. 73:106–113. https://doi.org/10.1016/j.plaphy.2013.08.011.

Chibuike GU, Obiora SC. 2014. Heavy metal polluted soils: Effect on plants and bioremediation methods. Applied and Environmental Soil Science. 2014:752708. https://doi.org/10.1155/2014/752708.

Chiroma TM, Ebewele RO, Hymore FK. 2014. Comparative assessment of heavy metal levels in soil, vegetables and urban grey waste water used for irrigation in Yola and Kano. International Refereed Journal of Engineering and Science. 3(2):1–9.

Dusengemungu L, Mubemba B, Gwanama C. 2022. Evaluation of heavy metal contamination in copper mine tailing soils of Kitwe and Mufulira, Zambia, for reclamation prospects. Scientific Reports. 12(1):11283. https://doi.org/10.1038/s41598-022-15458-2.

Ferreira SLC, Bezerra MA, Santos AS, dos Santos WNL, Novaes CG, de Oliveira OMC, Oliveira ML, Garcia RL. 2018. Atomic absorption spectrometry – A multi element technique. TrAC Trends in Analytical

Chemistry. 100(March 2018):1–6. https://doi.org/10.1016/j.trac.2017.12.012.

Gambus F, Wieczorek J. 2012. Pollution of fertilizers with heavy metals. Ecological Chemistry and Engineering. 19(4–5):353–360.

Guo G, Xiao J, Jeong BR. 2022. Iron source and medium pH affect nutrient uptake and pigment content in *Petunia hybrida* 'Madness Red' cultured In Vitro. International Journal of Molecular Sciences. 23(16):8943. https://doi.org/10.3390/ijms23168943.

Hansda A, Kumar V, Anshumali. 2015. Biosorption of copper by bacterial adsorbents: A review. Research Journal of Environmental Toxicology. 9(2):45–58. https://doi.org/10.3923/rjet.2015.45.58.

Herliana O, Soesanto L, Mawadah E. 2018. Phytobioremediation of cadmium-contaminated soil using combination of *Ipomoea reptans* Poir and *Trichoderma* sp. and its effect on spinach growth and yield. Journal of Degraded and Mining Lands Management. 6(1):1519–1526. https://doi.org/10.15243/jdmlm.2018.061.1519.

Jin Y, Luan Y, Ning Y, Wang L. 2018. Effects and mechanisms of microbial remediation of heavy metals in soil: A critical review. Applied Sciences. 8(8):1336. https://doi.org/10.3390/app8081336.

Kapahi M, Sachdeva S. 2019. Bioremediation options for heavy metal pollution. Journal of Health and Pollution. 9(24):191203. https://doi.org/10.5696/2156-9614-9.24.191203.

Kensa VM. 2011. Bioremediation – An overview. Journal of Industrial Pollution Control. 27:161–168.

Lajayer BA, Ghorbanpour M, Nikabadi S. 2017. Heavy metals in contaminated environment: Destiny of secondary metabolite biosynthesis, oxidative status and phytoextraction in medicinal plants. Ecotoxicology and Environmental Safety. 145:377–390. https://doi.org/10.1016/j.ecoenv.2017.07.035.

Li R-X, Cai F, Pang G, Shen Q-R, Li R, Chen W. 2015. Solubilisation of phosphate and micronutrients by *Trichoderma harzianum* and its relationship with the promotion of tomato plant growth. Lin W-X, editor. PLOS ONE. 10(6):e0130081. https://doi.org/10.1371/journal.pone.0130081.

Lindsay WL, Schwab AP. 1982. The chemistry of iron in soils and its availability to plants. Journal of Plant Nutrition. 5(4–7):821–840. https://doi.org/10.1080/01904168209363012.

Mimmo T, Del Buono D, Terzano R, Tomasi N, Vigani G, Crecchio C, Pinton R, Zocchi G, Cesco S. 2014. Rhizospheric organic compounds in the soil-microorganism-plant system: Their role in iron availability. European Journal of Soil Science. 65(5):629–642. https://doi.org/10.1111/ejss.12158.

Mishra GK. 2017. Microbes in heavy metal remediation: A review on current trends and patents. Recent Patents on Biotechnology. 11(3):188–196. https://doi.org/10.2174/1872208311666170120121025.

Myers W. [date unknown]. Iron toxicity. Myers Detox; [accessed 2022 June 2]. https://myersdetox.com/iron-toxicity/.

De Oliveira VH, Tibbett M. 2018. Cd and Zn interactions and toxicity in ectomycorrhizal basidiomycetes in axenic culture. PeerJ. 6:e4478. https://doi.org/10.7717/peerj.4478.

Redha AA. 2020. Removal of heavy metals from aqueous media by biosorption. Arab Journal of Basic and Applied Sciences. 27(1):183–193. https://doi.org/10.1080/25765299.2020.1756177.

Rout GR, Sahoo S. 2015. Role of iron in plant growth and metabolism. Reviews in Agricultural Science. 3:1–24. https://doi.org/10.7831/ras.3.1.

Shiwachi H, Kikuno H, Okonkwo CC, Asiedu R. 2006. Iron toxicity symptoms in yams (*Dioscorea* spp.) grown in water culture. Tropical Science. 46(3):160–165. https://doi.org/10.1002/ts.172.

Shrivastava P, Kumar R. 2015. Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. Saudi Journal of Biological Sciences. 22(2):123–131. https://doi.org/10.1016/j.sjbs.2014.12.001.

Soesanto L, Ilahiyyah H, Mugiastuti E, Manan A, Rostaman R. 2020. Raw secondary metabolites of *Trichoderma harzianum* T10 in tapioca flour towards cucumber damping-off. Biosaintifika: Journal of Biology & Biology Education. 12(2):226–234. https://doi.org/10.15294/biosaintifika.v12i2.24567.

Soesanto L, Sastyawan MWR, Nurliana N, Mugiastuti E. 2022. Potency of two *Trichoderma harzianum* isolates in liquid and solid organic formula for controlling bacterial wilt on tomato in the field. Jurnal Hama dan Penyakit Tumbuhan Tropika. 22(2):116–125. https://doi.org/10.23960/jhptt.222116-125.

Soesanto L, Sudarmono S, Prihatiningsih N, Manan A, Iriani E, Pramono J. 2005. Potensi agensia hayati dan nabati dalam mengendalikan penyakit busuk rimpang jahe [Potential of biological and plant-based agents in controlling ginger rhizome rot disease]. Jurnal Hama dan Penyakit Tumbuhan Tropika. 5(1):50-57. Indonesian. https://doi.org/10.23960/j.hptt.1550-57.

Strezov V, Chaudhary C. 2017. Impacts of iron and steelmaking facilities on soil quality. Journal of Environmental Management. 203:1158–1162. https://doi.org/10.1016/j.jenvman.2017.02.040.

Vinale F, Ghisalberti EL, Sivasithamparam K, Marra R, Ritieni A, Ferracane R, Woo S, Lorito M. 2009. Factors affecting the production of *Trichoderma harzianum* secondary metabolites during the interaction with different plant pathogens. Letters in Applied Microbiology. 48(6):705–711. https://doi.org/10.1111/j.1472-765X.2009.02599.x.

Wang P, Chao D. 2020. Phytoremediation of heavy metal contamination and related molecular

mechanisms in plants. Shengwu Gongcheng Xuebao/Chinese Journal of Biotechnology. 36(3):426–435. https://doi.org/10.13345/j.cjb.190332.

Yan A, Wang Y, Tan SN, Mohd Yusof ML, Ghosh S, Chen Z. 2020. Phytoremediation: A promising approach for revegetation of heavy metal-polluted land. Frontiers in Plant Science. 11:359. https://doi.org/10.3389/fpls.2020.00359.

Zahra N, Hafeez MB, Shaukat K, Wahid A, Hasanuzzaman M. 2021. Fe toxicity in plants: Impacts and remediation. Physiologia Plantarum. 173(1):201–222. https://doi.org/10.1111/ppl.13361.

Zhou J, Zeng Y, Demopoulos GP, Li C, Li Z. 2018. Phase transition of FeSO 4 ·7H 2 O to FeSO 4 ·H 2 O in the H 2 SO 4 –HCl–H 2 O system by modeling solubility. ACS Sustainable Chemistry & Engineering. 6(2):2207–2219. https://doi.org/10.1021/acssuschemeng.7b03627.