Development of an effective bioprocess for fast production of enriched biocompost from municipal solid wastes

By:
Gholamreza Salehi Jouzani
Microbial Biotechnology and Biosafety Department
Agricultural Biotechnology Research Institute of Iran (ABRII)

Co-authors:
Helen Pourmazaheri, Ebrahim Karimi, Meisam Tabatabaei, Reza Maali Amiri

Agricultural and forestry residue in Iran:

about 100 million tones

6 mtns rice straw without any application!!!!!!

Ranjan A. and S Moholkar, 2011, Fuel

Municipal Solid Waste in Iran

- ✓MSW in Iran: 20 million tones per year
- ✓MSW per Capita per day: 0.7 kg (70% organic and 30% inorganic)
- ✓84% landfilling, 10% composting, 6% Recycling

Ref: Nabizadeh et al., 2008

The most important common problems in the conventional compost production process (especially in Iran):

- ✓Long time period of the process (3-6 months) and therefore, low cost efficiency
- ✓Immaturity of the final produced compost
- ✓Bad odor of the final produced compost
- ✓Presence of plant and human pathogens
- ✓Presence of heavy metals and other toxic materials

Importance of Microbes in composting

As composting is a biological decomposition process, so the most important factor affecting the compost quality is type and quantity of microorganisms (mesophilic and thermophilic bacteria especially actinomycetes and fungi) present in the three different phases of the process (Novinscak et al., 2007; Vargas-Garcia et al., 2010).

The objectives of the present study

1. Isolation and characterization of effective microorganisms during composting process of MSW and agricultural residues

2. Designing a bioprocessing system to reduce the period of compost production and to produce enriched compost by using the microbial cocktail and optimization Carbon to Nitrogen ratio

Isolation of the effective microorganisms

- **First Strategy:** isolation from conventional composting process at the compost plants based on the conventional microbiological procedures (Gregersen., 1978; Schaad et al., 2004; Bergey et al., 1986).

- **The second strategy:** Biocomposting of agricultural residues, such as sugarcane baggase, rice straw and wheat straw in a new designed bioreactor (to simulate compost process at laboratory scale)

Designing a Dynamic Solid State bioreactor for simulation of biocomposting process for isolation of effective microorganisms (With automatic temperature/air flow/relative moisture and mixing rate controlling systems)

Process of isolation and selection of effective microorganisms

Rice straw

Wheat straw

Baggase

MSW

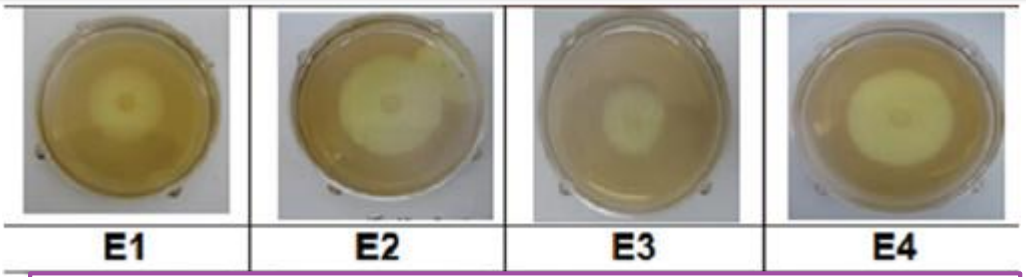
Solid state fermentation

Conventional industrial composting

Mesophilic / Thermophilic Fungi

Mesophilic / Thermophilic Bacteria

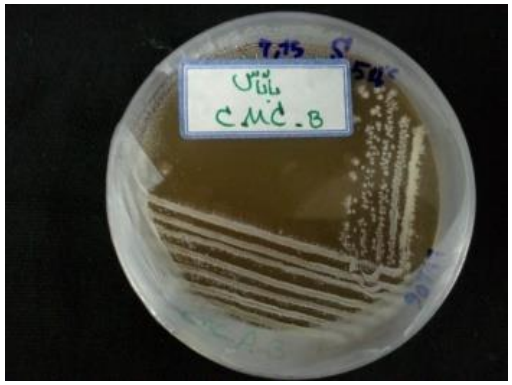
Enrichment and Selective plate–screening Methods

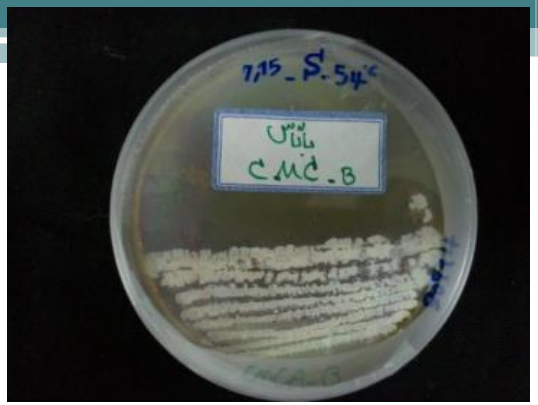


Cellulose, Cellubiose, xylane, starch

ABRII

Growth on the selective carbon sources

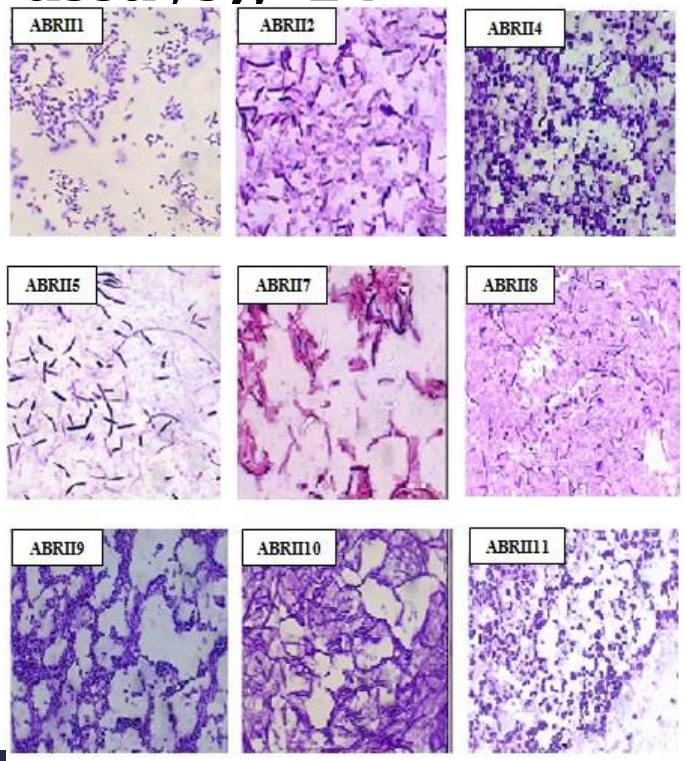




About 300 bacterial and fungal isolates were isolated from different sources

Based on the Enzyme activity tests (Qualitative and Quantitative assays), 14 isolates were selected

Cellulase activity (CMC)
Protease activity (Skim milk)
Amylase activity (Starch)
Lipase activity (tween 80)
Xylanase activity (Xylane)



Enzyme Activity

Enzymes Strains	Protease (Unit/ml)	Cellulase (mUnit/ml)	Amylase (mUnit/ml)	Xylanase (mUnit/ml)	Lipase (mUnit/ml
ABRII1	4.31 a	40 c	13.15 c	121.94 a,b,	121.2a,
ABRII2	4.50 a	50 b	18.3 c	62.66 d	118a,
ABRII3	4.53 a	50b	12.77 cd	95.94 c	15.8d
ABRII4	4.16 a	40 b	18.09 c	110.89 b	22d
ABRII5	4.63 a	55 b	13.89 cd	96.75 c	75.4b
ABRII6	2.34 b	40 c	17.9 c,	112.35 b	14d
ABRII7	4.03 a,b	55 b	13.05 cd	114.62 b	80b
ABRII8	4.42 a	50 b	80.54 b	114.95 b	77.6b
ABRII9	2.24 b	50 b	8.67 d	124.78 a,b	45c
ABRII10	2.24 b	110 a	163.78 a	130.30 a	42.3c
ABRII11	2.25 b	46 bc	12.03 cd	133.31 a	48c

Molecular Identification of the selected strains

DNA Extraction:

Genomic DNA extraction kit according to the manufacturer's (BIONEER) instruction.

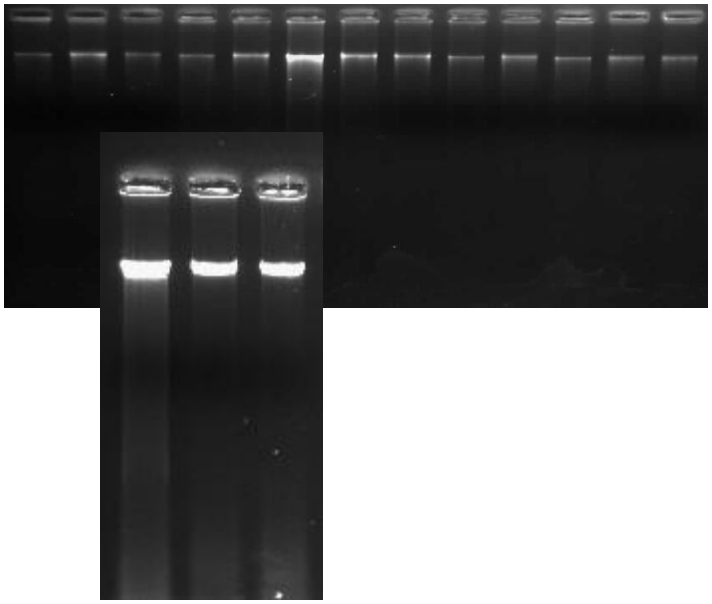
PCR:

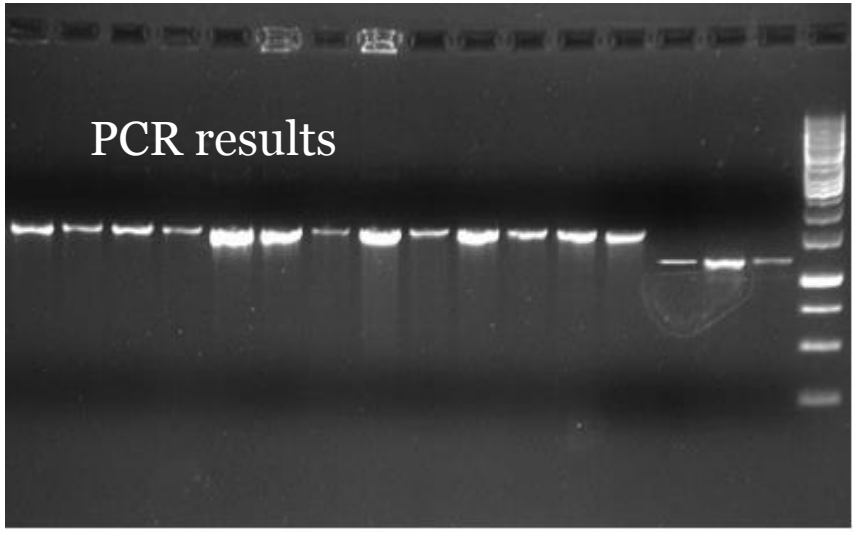
16SrDNA primers for bacteria

PA-F (5'-GAGTTTGATCCTGGCTCAG-3') and PA-R (5'-AAGGAGGTGATCCAGCCGCA-3') were used (Zakaria et al., 2010).

18SrDNA primers for fungal strains

M13 (F)	5'-TAAAACGACGGCCAG-3'
M13 (R)	5'-CAGGAAACAGCTATGAC-3'





The selected strains

Species and Subspecies	Strains	Accession No.
Aneurinibacillus migulanus	ABRII1	JN252029
Brevibacillus parabrevis	ABRII2	JN315628
Pseudoxanthomonas suwonensis	ABRII3	JN315629
Brevibacillus formosus	ABRII4	JN315630
Bordetella petrii	ABRII5	JN315631
Bacillus licheniformis	ABRII6	JN315632
Bacillus licheniformis	ABRII7	JN315633
Geobacillus thermodenitrificans	ABRII8	JN315634
Geobacillus sp.	ABRII9	JN315635
[illegible]	ABRII10	JN315636
Aspergillus fumigatus	ABRII12	-
Aspergillus fumigatus	ABRII13	-
Aspergillus fumigatus	ABRII14	-

Growth conditions of the selected strains for biomass production

Microbial Strains	pH	Temperature	Culture media	Time
Aneurinibacillus migulanus	7	30	NB	24h
Brevibacillus parabrevis	7	30	NB	24h
Pseudoxanthomoas suwonensis	7.3	30	TSB	24h
Brevibacillus formosus	7	30	NB	24h
Bordetella petrii	7.3	30	TSB	24h
Bacillus licheniformis	7	37	NB	24h
Bacillus licheniformis	7	37	NB	24h
Geobacillus thermodenitrificans	7	60	NB	24h
Geobacillus sp	7	50	NB	24h
Thermoactinomyces intermedius	7.3	55	TSB	24h
Brevibacillus agri	7	30	NB	24h

Growth conditions of the selected strains for biomass production

Strain	Microbial Strains	pH	Temperature	Culture media	Time
ABRII12	***Aspergillus fumigatus***	5.1	37	NB	24h
ABRII13	***Aspergillus fumigatus***	7	37	NB	24h
ABRII14	***Aspergillus fumigatus***	7.3	37	TSB	24h

Evaluation of the effect of microbial cocktail on the composting process at pilot scale

Treatments

- ✓ NC (Normal Composting: using only MSW)
- ✓ NC+ Wood chips (MSW+W)
- ✓ NC+ microbial inoculi+ wood chips (MSW+W+M)

The experiments were performed in the Compost Plant of Isfahan City

Mass production of the selected strains

The strains were cultured in different media and temperatures in advanced 10 lit Fermentor (New Brunswick Scientific Co., INC. Edison, N.J., BIOFLO 2000, USA).

Experimental setup

Windrows: height: 1 .5m, Width: 2 m and length: 10 m)

Preparation of wood Chips/agricultural residues (C/N up to 26)

Preparation of MSW

Mixing MSW and Agricultural residues

Experimental setup

Addition of Microbial Cocktail
(10 liters (CFU=10^9)/ton biomass)

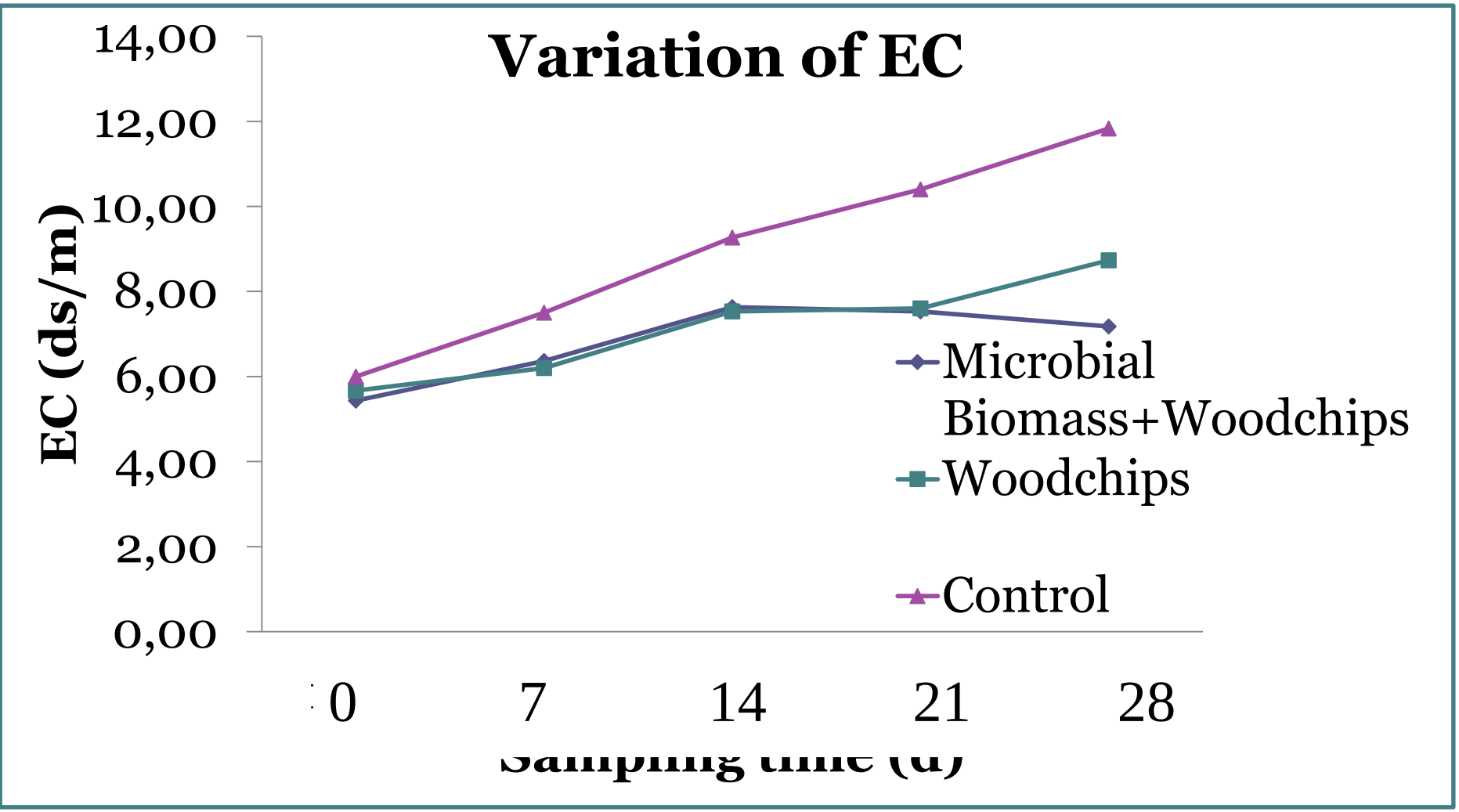
Mixing and turning (Aeration)

Moisture optimization

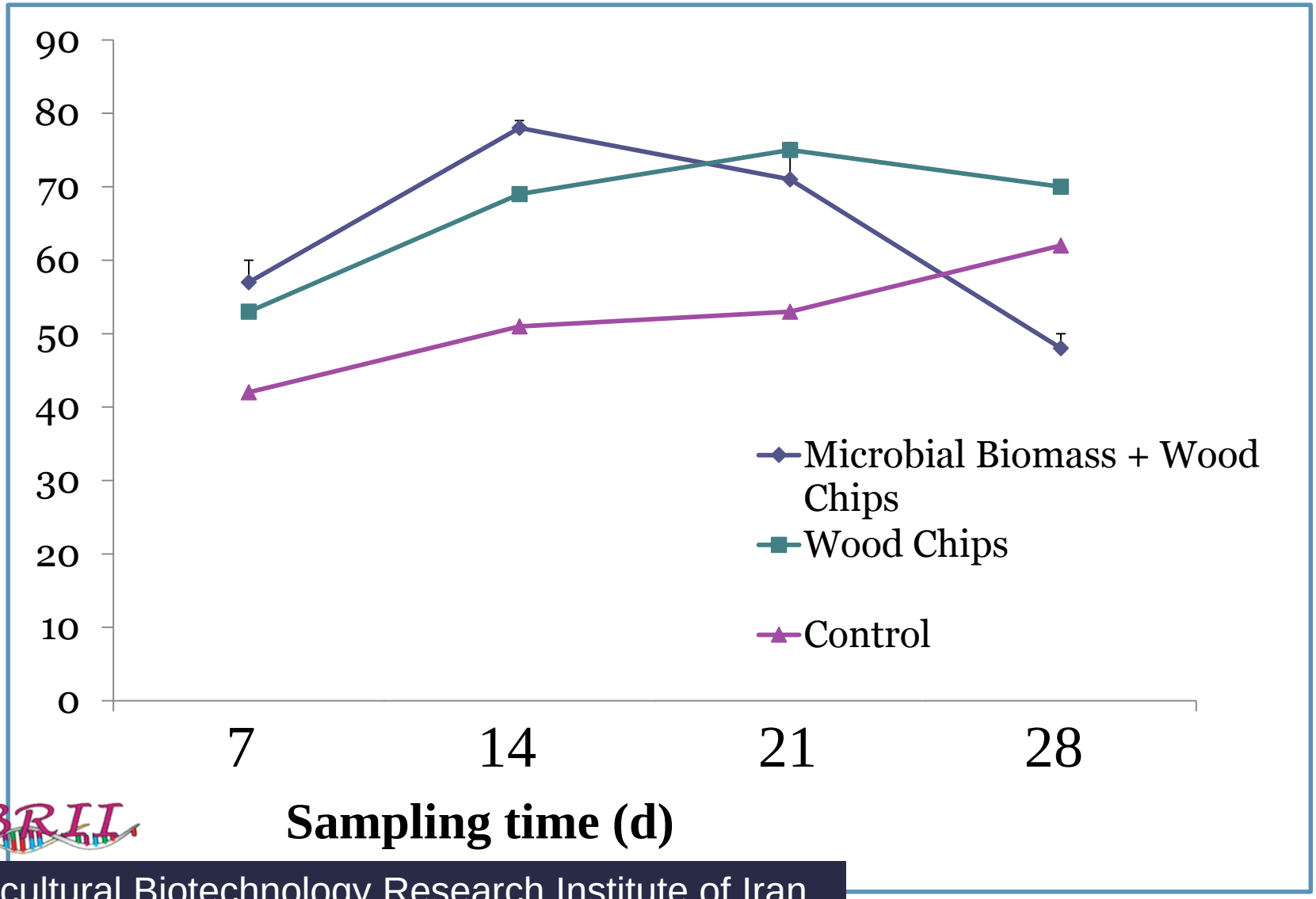
Samplings and analyses during the process

Samples were taken on 0, 7, 14, 21 and 28 days based on Composite method (Crecchio et al., 2001)

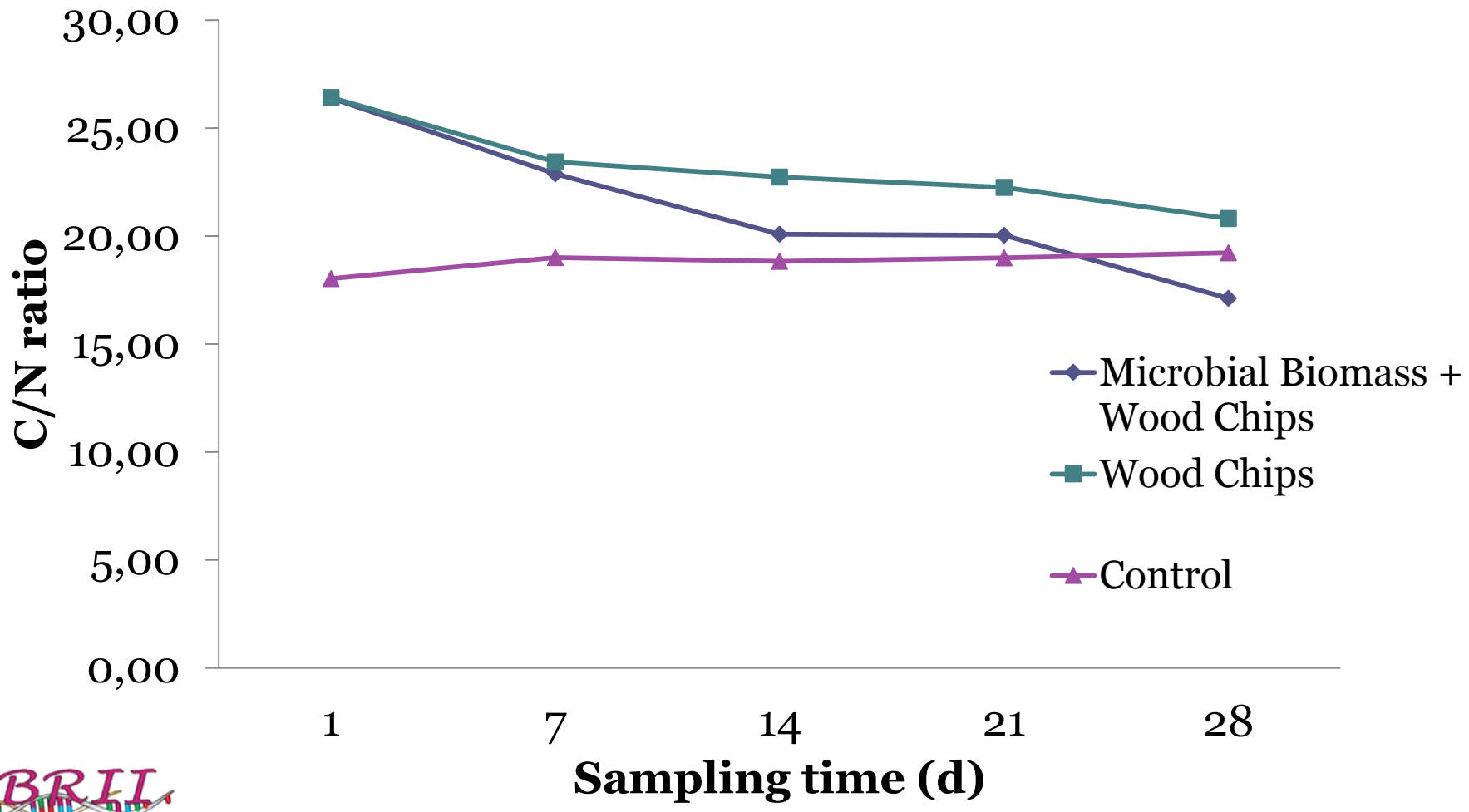
- Moisture content
- Temperature
- C/N
- pH
- Heavy metals
- Plant and human pathogens
- Germination test
- Cation content

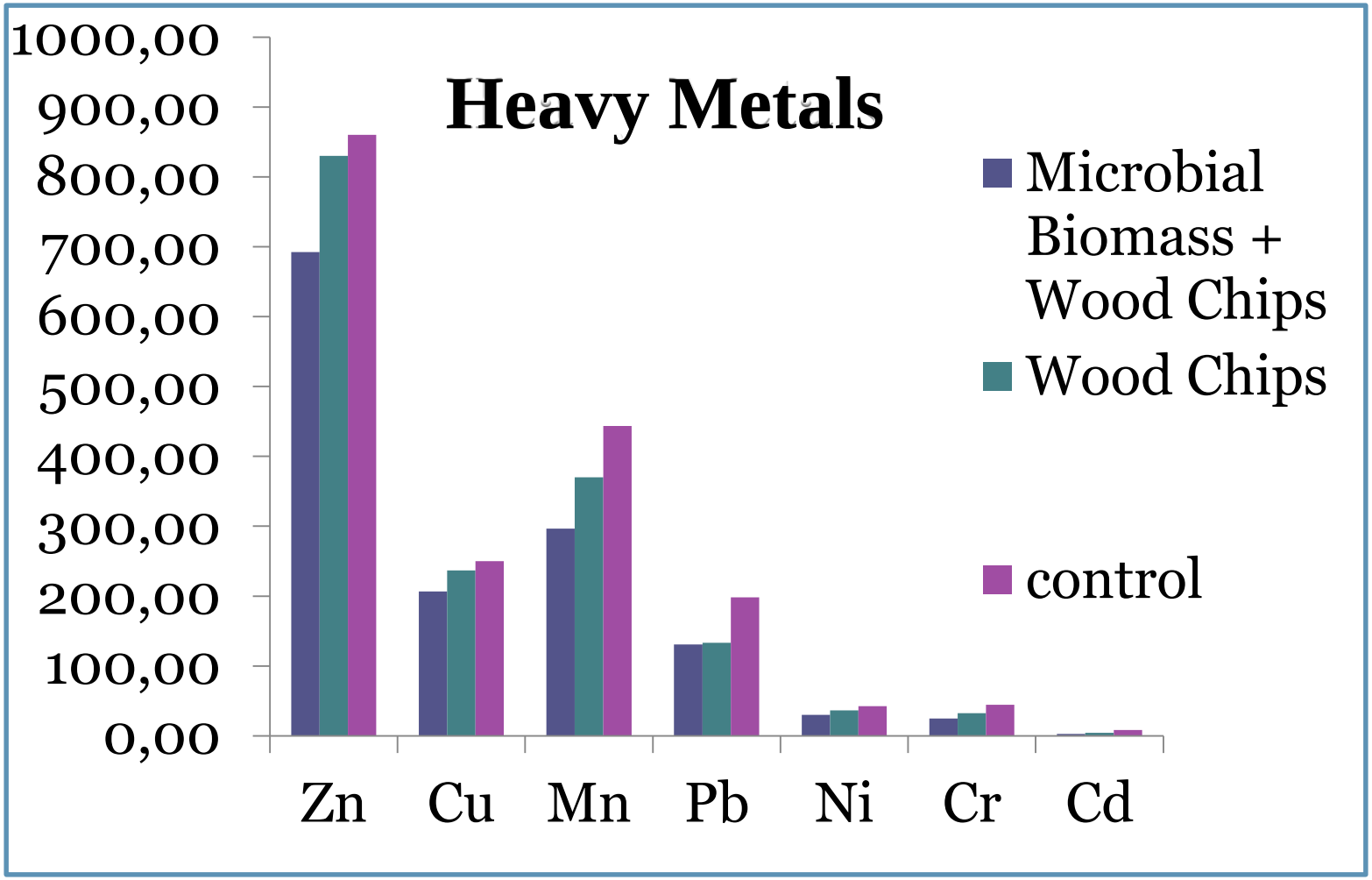

Variation of EC
14,00
12,00
10,00
8,00
6,00
4,00
2,00
0,00
EC (ds/m)
0
7
14
21
28
Microbial Biomass+Woodchips
Woodchips
Control

Temperature profile during the process



Variation of C/N ratio

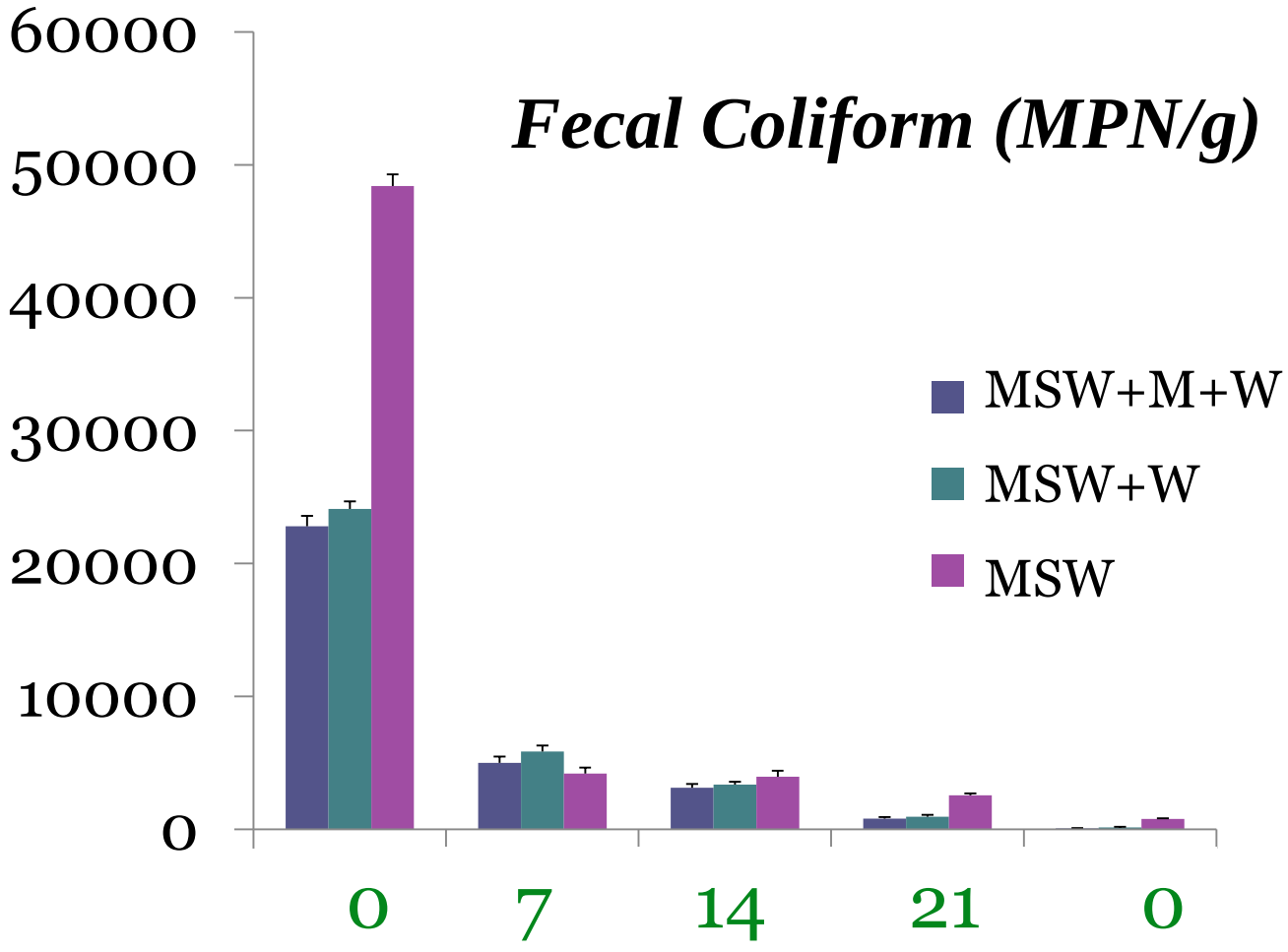




US EPA (2009: 1992) Standard

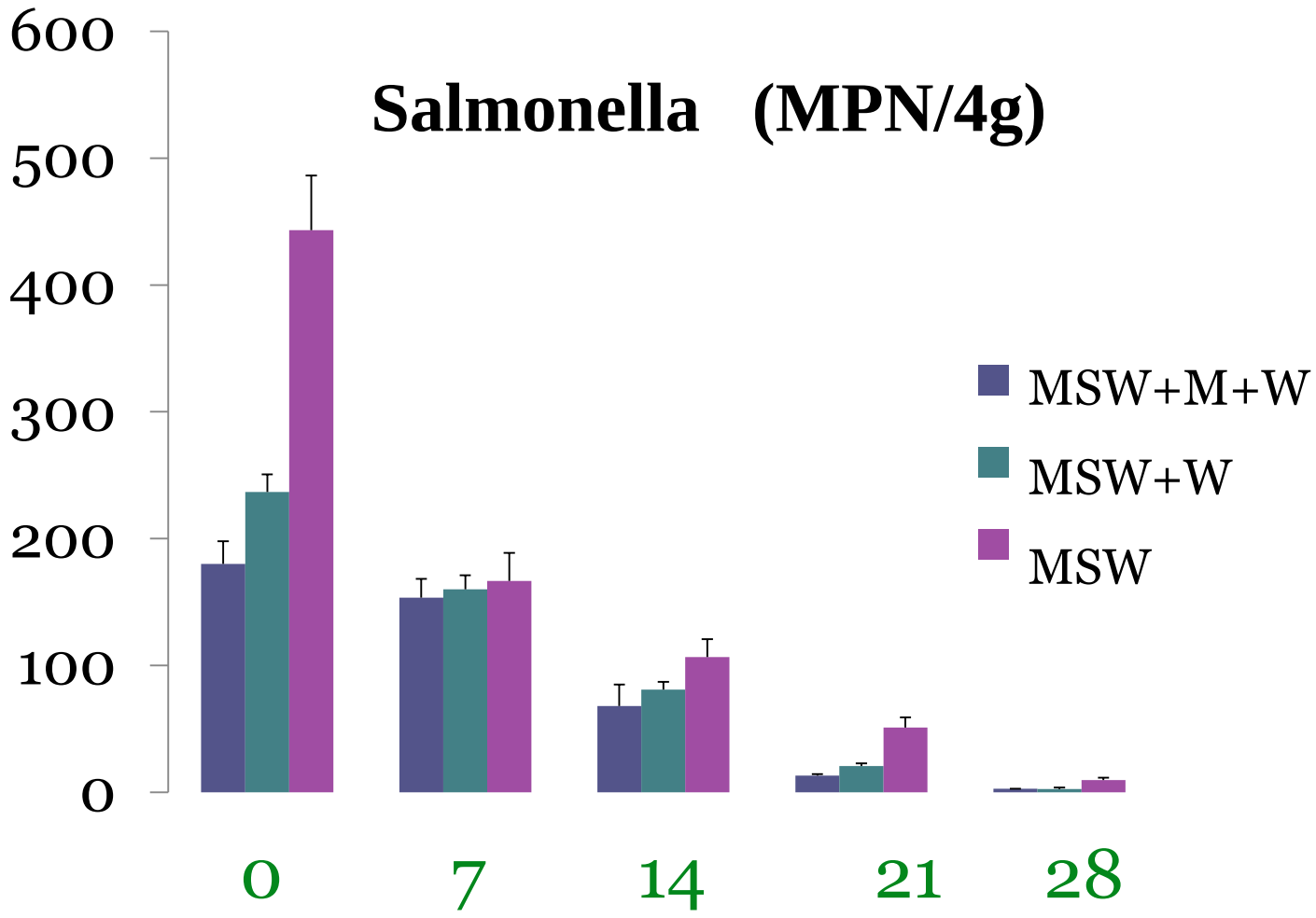
UK PAS 100 (2011) Standard for Heavy Metals in Compost

Presence of Human pathogens



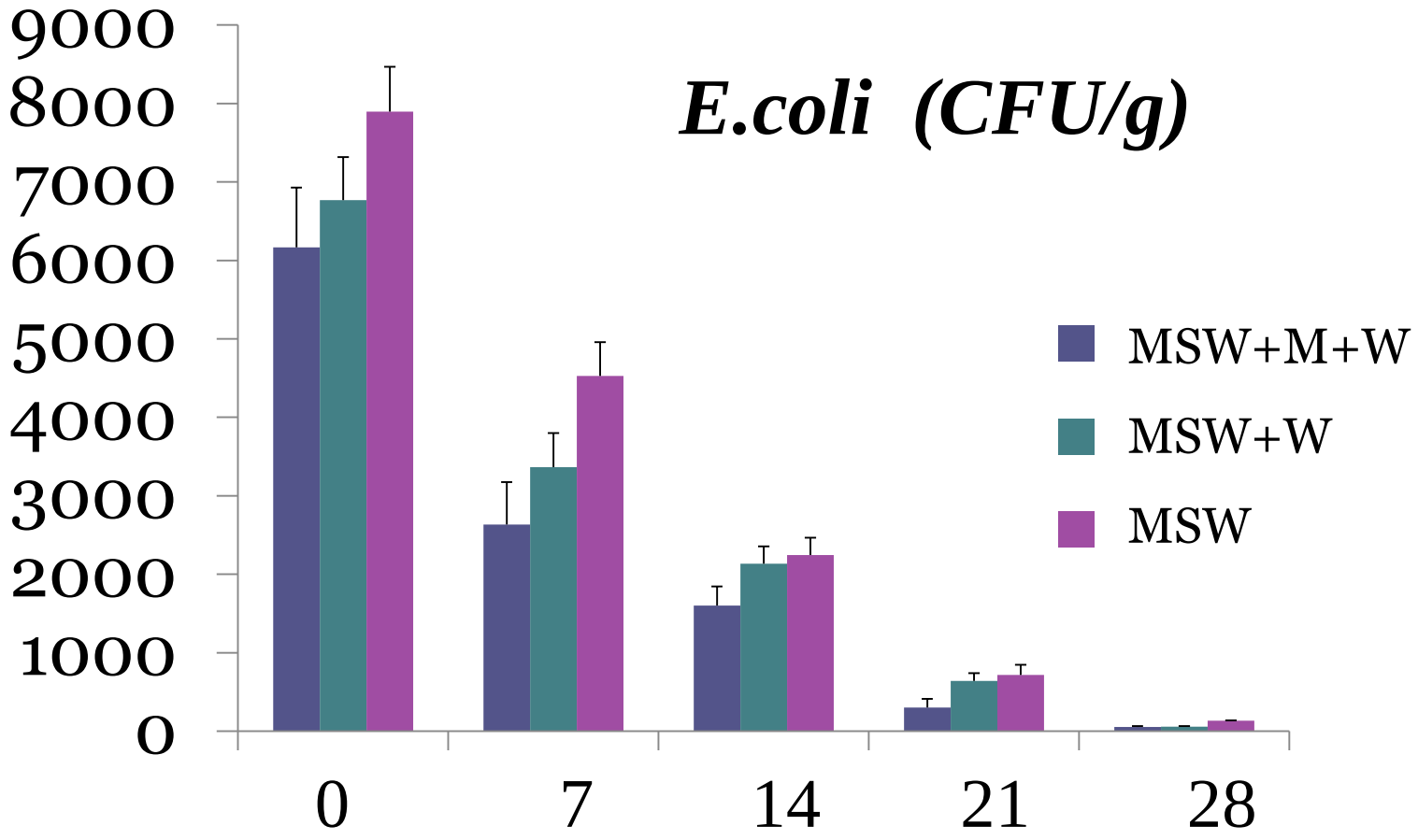
UK PAS 100 (2011) Standard for Pathogens in Compost

Presence of Human pathogens



UK PAS 100 (2011) Standard for Pathogens in Compost

Presence of Human pathogens

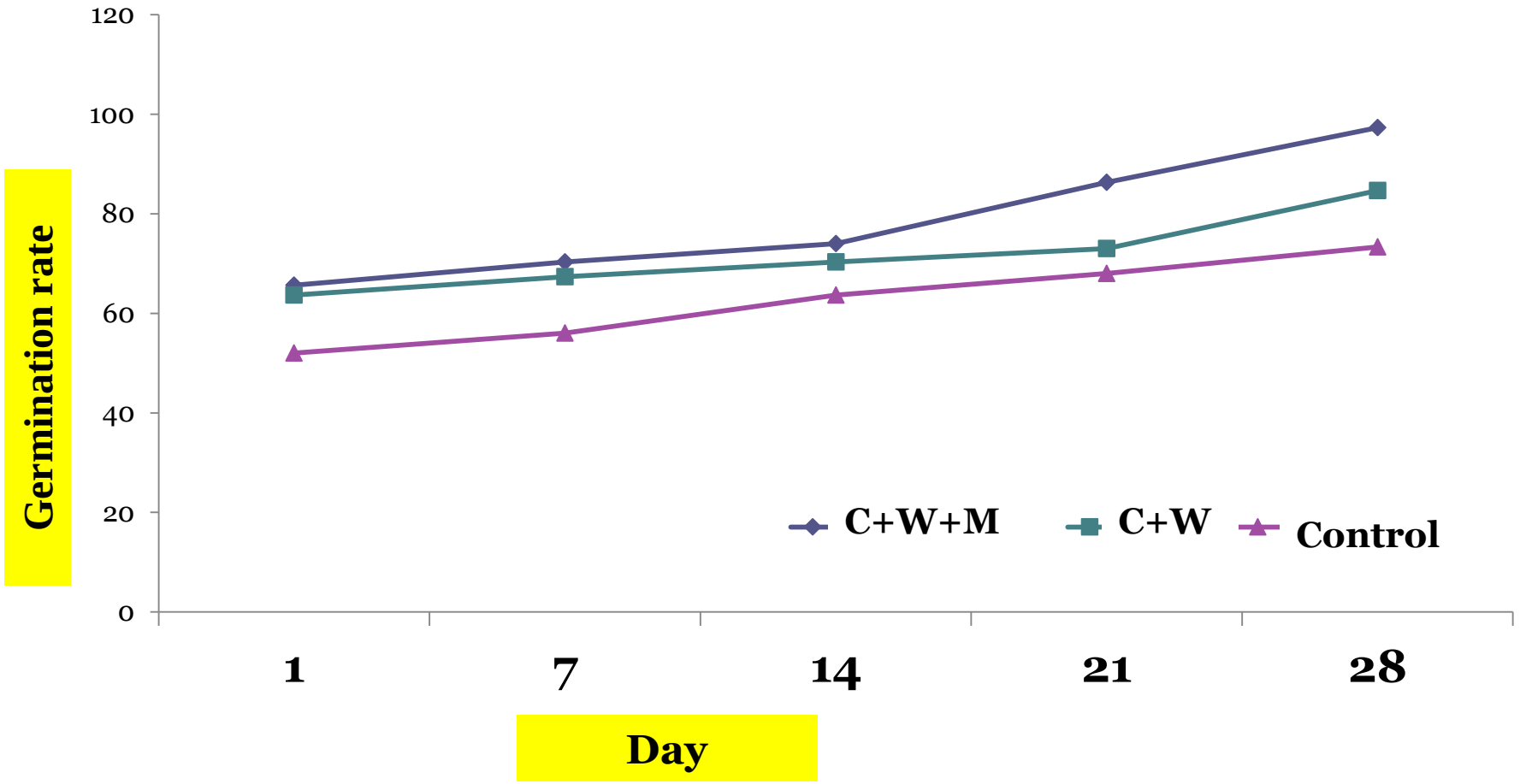


UK PAS 100 (2011) Standard for Pathogens in Compost

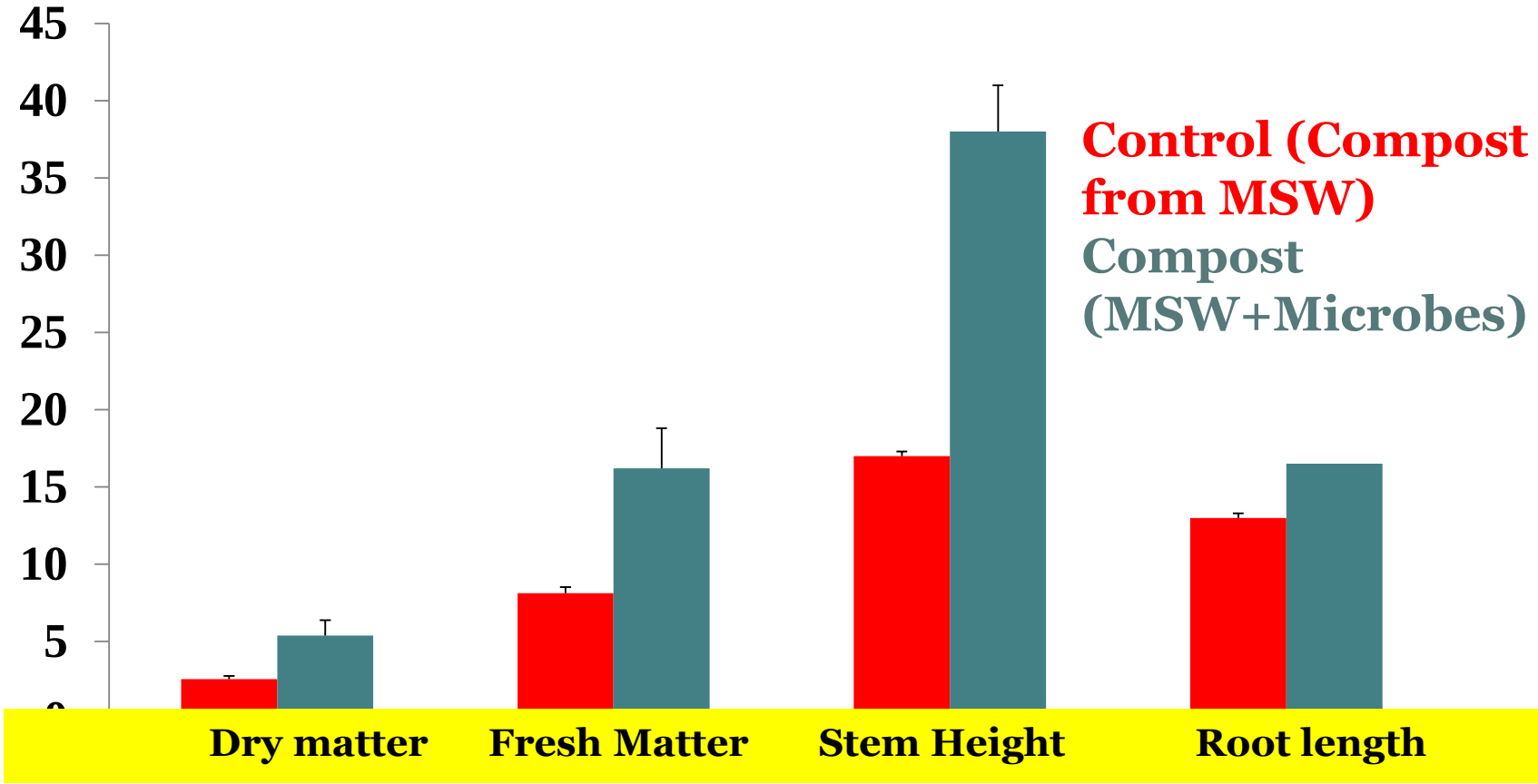
Evaluation of the plant pathogens populations

Plant Pathogens	Phytophthora	Pythium	Rhizoctonia
C+W+M	0	0	0
C+W	14	0	0
C	22	0	15

Germination Test



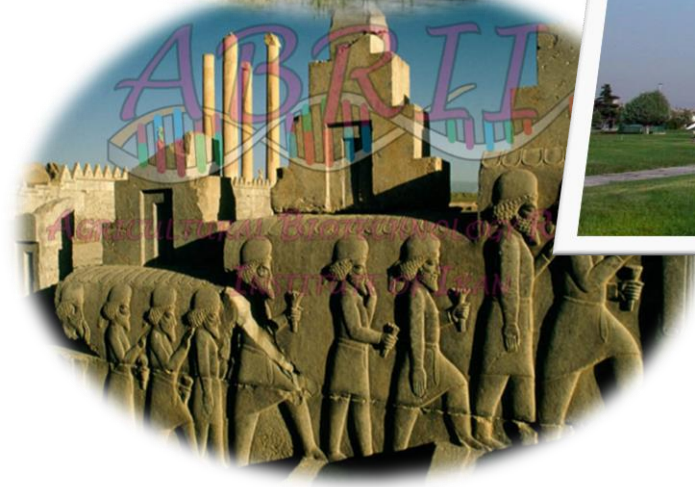
The effect of produced biocompost on the biomass indexes of the wheat



CONCLUDING REMARKS

- **Fourteen native effective bacterial and fungal strains with high enzyme activities were identified and characterized**
- **Pilot production of compost from MSW using microbial cocktail and agricultural residues (wood chips) was performed.**
- **The composting process was performed upto28 days, and the quality of the produced compost (C/N, heavy metal contents, pathogens, toxicity for seeds) was compatible with the national and international standards.**

Thank you for your attention



Some beautiful places in Iran