Theme Number: 3 **Sub-topic:** 3.2 Energy from waste and alternative resources

Study on the biodegradation of phenol derivatives by oleaginous yeast *Rhodosporidium toruloides* 9564^T *and* effect of heavy metals on phenol degradation

Sangeeta Singh[a], and Kasturi Dutta[a*]

[a] National Institute of Technology Rourkela, Odisha 769008, India (Email:*duttakasturi@gmail.com; duttakasturi@nitrkl.ac.in*)

Corresponding author
Dr. Kasturi Dutta, Department of Biotechnology and Medical Engineering, National Institute of Technology Rourkela, Odisha, 769008, India **(**Email:*duttakasturi@gmail.com;duttakasturi@nitrkl.ac.in)*

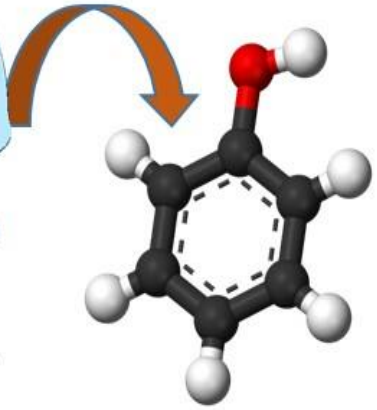
Abstract
An increase in industrialization has led to the indiscriminate discharge of concentrated harmful chemical pollutants into the environment, which directly impacts the nature. Heavy metals and phenol derivatives are present in industrial effluent, making their remediation challenging. The use of oleaginous yeast *Rhodosporidium toruloides* 9564^T in the treatment of wastewater containing phenol and its derivative is a research focus. The oleaginous yeast can resist heavy metals and degrade phenol and its derivative effectively. Experiments were designed using simulated wastewater by varying 4-chlorophenol and catechol concentration in the range of 0.25 – 1.5 g/L with an inoculum size of 10%. The oleaginous yeast *R. toruloides* 9564^T was found to completely degrade 4-chlorophenol upto 0.75 g/L and catechol upto 1 g/L. The effect of heavy metal chromium (Cr) in the range of 5-25 mg/L on phenol degradation was also studied using the yeast, and the obtained result confirmed that above 5 mg/L of chromium had a significant impact on phenol degradation by extending the degradation period from 36 h to 108 h in 0.5 g/L phenol concentration. The maximum biomass and lipid content obtained from 4-chlorophenol and catechol degradation were 2.38 g/L and 4.03 g/L, and lipid content was 28.37 and 34.6% respectively. After complete degradation of phenol derivatives, the samples were used for the toxicological study. Therefore, the use of oleaginous yeast *R. toruloides* 9564^T for treatment of phenolic wastewater and lipid production could be an economical approach towards biodiesel production.

Keywords: Biodiesel production, Biomass, Catechol, 4-Chlorophenol, Chromium, Heavy metal, Lipid content

Study on the biodegradation of phenol derivatives by oleaginous yeast *Rhodosporidium toruloides* 9564^{T} *and* effect of heavy metals on phenol degradation

Presented by: Sangeeta Singh
National institute of
Technology Rourkela India

INTRODUCTION



Phenol and derivatives containing wastewater effluents are release from paper and pulp, dye, coal, pharmaceuticals, petroleum refineries etc.

Hazardous to aquatic and terrestrial life

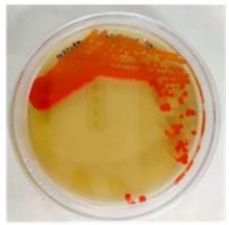
Toxic effects severe skin damage, muscle pains, gastrointestinal disorders, renal failure, peripheral nerve damage, cancer

Highly corrosive

Removal or degradation of phenol and its derivative from water is necessary

- Every year these industries release from 1mg/L to 7000 mg/L phenol and there derivatives in their wastewater effluents.
- Permisible limit of chromium in drinking water was 0.05 mg/L declared by WHO

PREVIOUS WORK

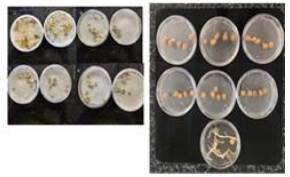


R. toruloides growth in agar plate

Seed culture Preparation

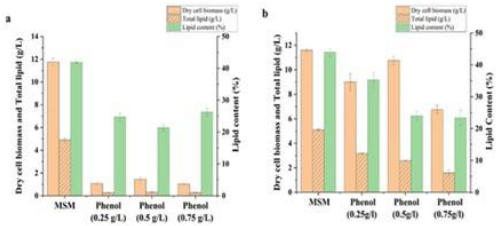
MSM media inoculated with seed culture

Growth of *R toruloides* in Phenolic media

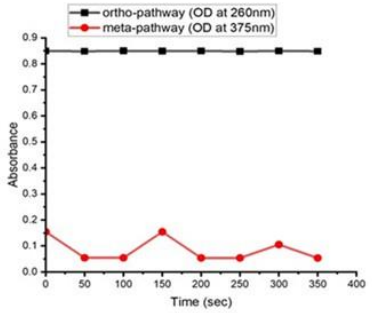


Toxicological study

Phenol estimation by 4 aminoantipyrine method

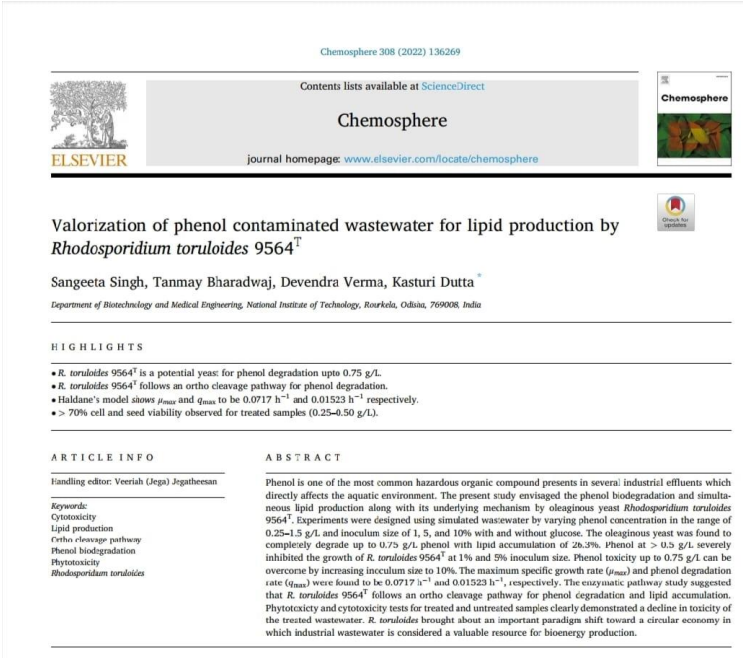


Lipid accumulation



Enzymatic method for pathway determination

Complete degradation of phenol upto 0.75 g/L by using oleaginous yeast *R. toruloides*.



Chemosphere 308 (2022) 136269

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Valorization of phenol contaminated wastewater for lipid production by *Rhodosporidium toruloides* 9564^T

Sangeeta Singh, Tanmay Bharadwaj, Devendra Verma, Kasturi Dutta *

Department of Biotechnology and Medical Engineering, National Institute of Technology, Rourkela, Odisha, 769008, India

HIGHLIGHTS

- *R. toruloides* 9564^T is a potential yeast for phenol degradation upto 0.75 g/L.
- *R. toruloides* 9564^T follows an ortho cleavage pathway for phenol degradation.
- Haldane's model shows μ_{max} and q_{max} to be 0.0717 h^{-1} and 0.01523 h^{-1} respectively.
- > 70% cell and seed viability observed for treated samples (0.25–0.50 g/L).

ARTICLE INFO

Handling editor: Veeriah (Jega) Jegatheesan

Keywords:
Cytotoxicity
Lipid production
Ortho cleavage pathway
Phenol biodegradation
Phytotoxicity
Rhodosporidium toruloides

ABSTRACT

Phenol is one of the most common hazardous organic compound presents in several industrial effluents which directly affects the aquatic environment. The present study envisaged the phenol biodegradation and simultaneous lipid production along with its underlying mechanism by oleaginous yeast *Rhodosporidium toruloides* 9564^T. Experiments were designed using simulated wastewater by varying phenol concentration in the range of 0.25–1.5 g/L and inoculum size of 1, 5, and 10% with and without glucose. The oleaginous yeast was found to completely degrade up to 0.75 g/L phenol with lipid accumulation of 26.3%. Phenol at > 0.5 g/L severely inhibited the growth of *R. toruloides* 9564^T at 1% and 5% inoculum size. Phenol toxicity up to 0.75 g/L can be overcome by increasing inoculum size to 10%. The maximum specific growth rate (μ_{max}) and phenol degradation rate (q_{max}) were found to be 0.0717 h^{-1} and 0.01523 h^{-1}, respectively. The enzymatic pathway study suggested that *R. toruloides* 9564^T follows an ortho cleavage pathway for phenol degradation and lipid accumulation. Phytotoxicity and cytotoxicity tests for treated and untreated samples clearly demonstrated a decline in toxicity of the treated wastewater. *R. toruloides* brought about an important paradigm shift toward a circular economy in which industrial wastewater is considered a valuable resource for bioenergy production.

METHODOLOGY

- Growth of *R. toruloides* in MSM containing different phenol derivatives (Catechol, 4-chlorophenol and 4- nitrophenol)
- Growth of *R. toruloides* in MSM containing different phenol and chromium.

- Dry biomass, total lipid and Lipid content estimation in different phenol derivative media
- Dry biomass, total lipid and Lipid content estimation in different phenol and chromium containing MSM media.

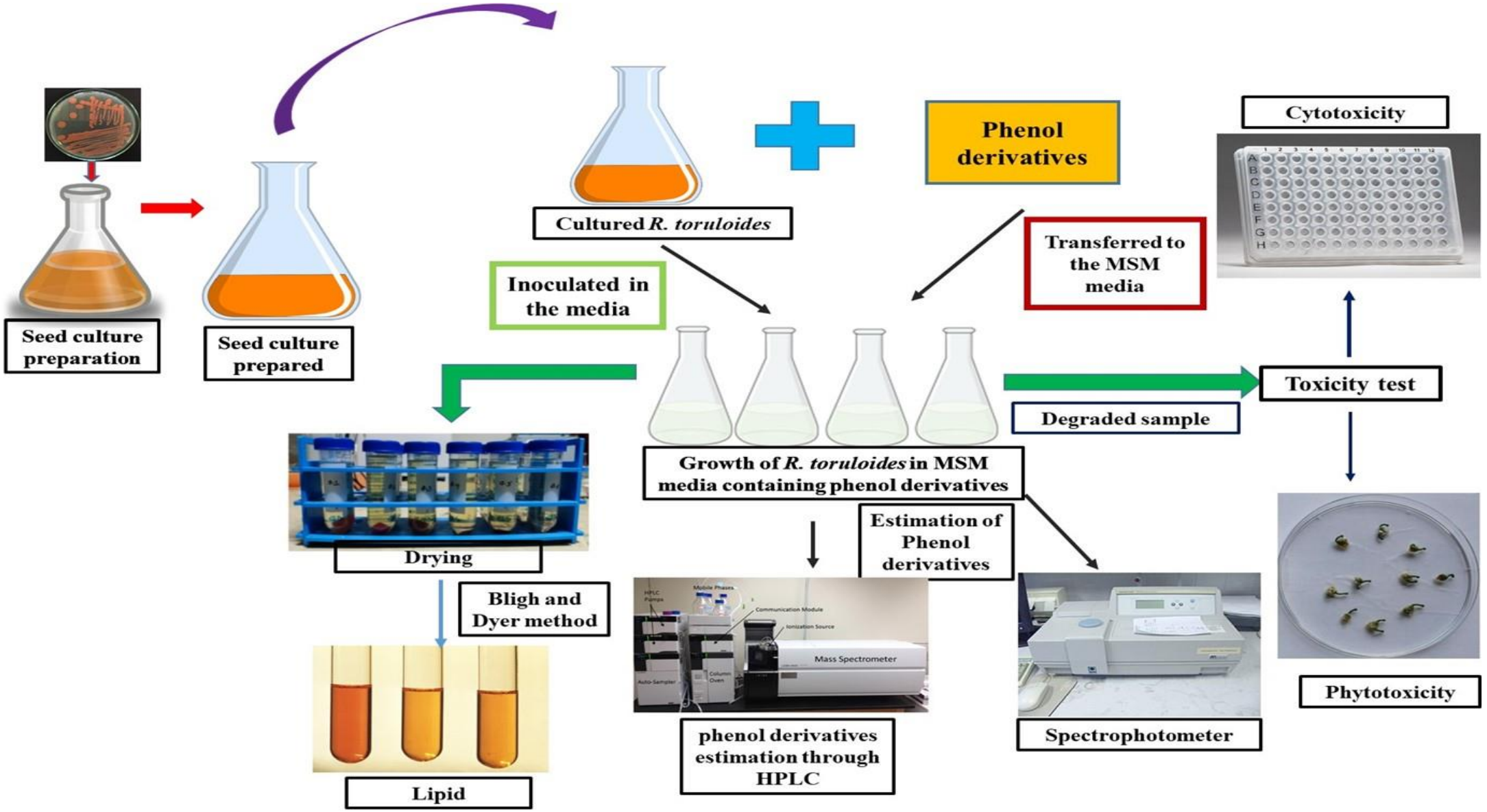
- Cell morphology analysis in different media (YPED, MSM, MSM+Phenol and MSM+Phenol+Chromium)

- Cell size and presence of lipid droplet analysis using confocal.

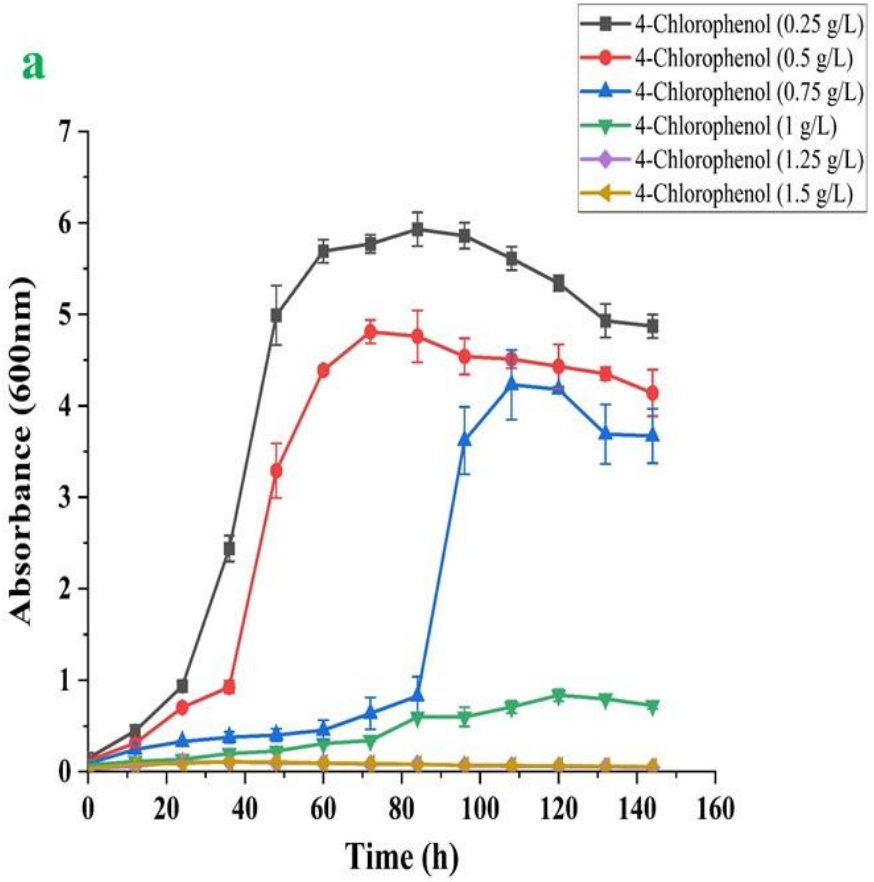
Toxicological study for degraded sample:

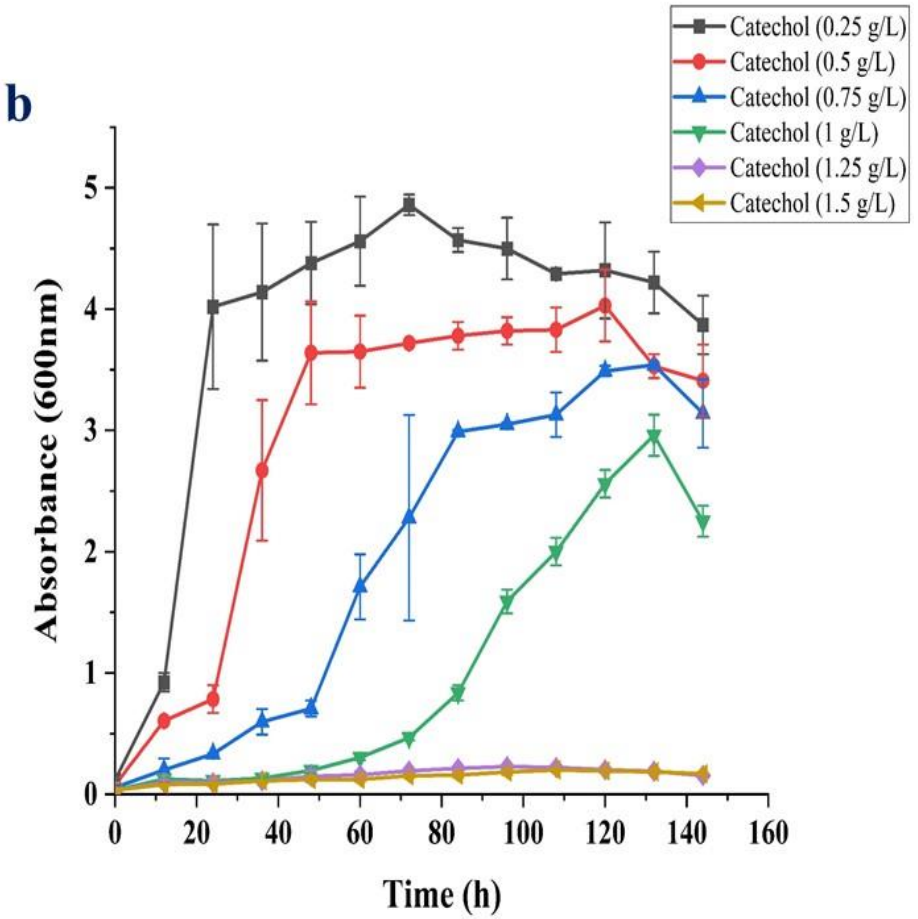
- Phytotoxicity
- Cytotoxicity

STEPS


Seed culture preparation
Seed culture prepared
Cultured R. toruloides
Phenol derivatives
Inoculated in the media
Transferred to the MSM media
Cytotoxicity
Growth of R. toruloides in MSM media containing phenol derivatives
Degraded sample
Toxicity test
Drying
Bligh and Dyer method
Estimation of Phenol derivatives
Phytotoxicity
Lipid
phenol derivatives estimation through HPLC
Spectrophotometer

RESULTS: Phenol derivatives degradation using *R. toruloides* 9564^{T}



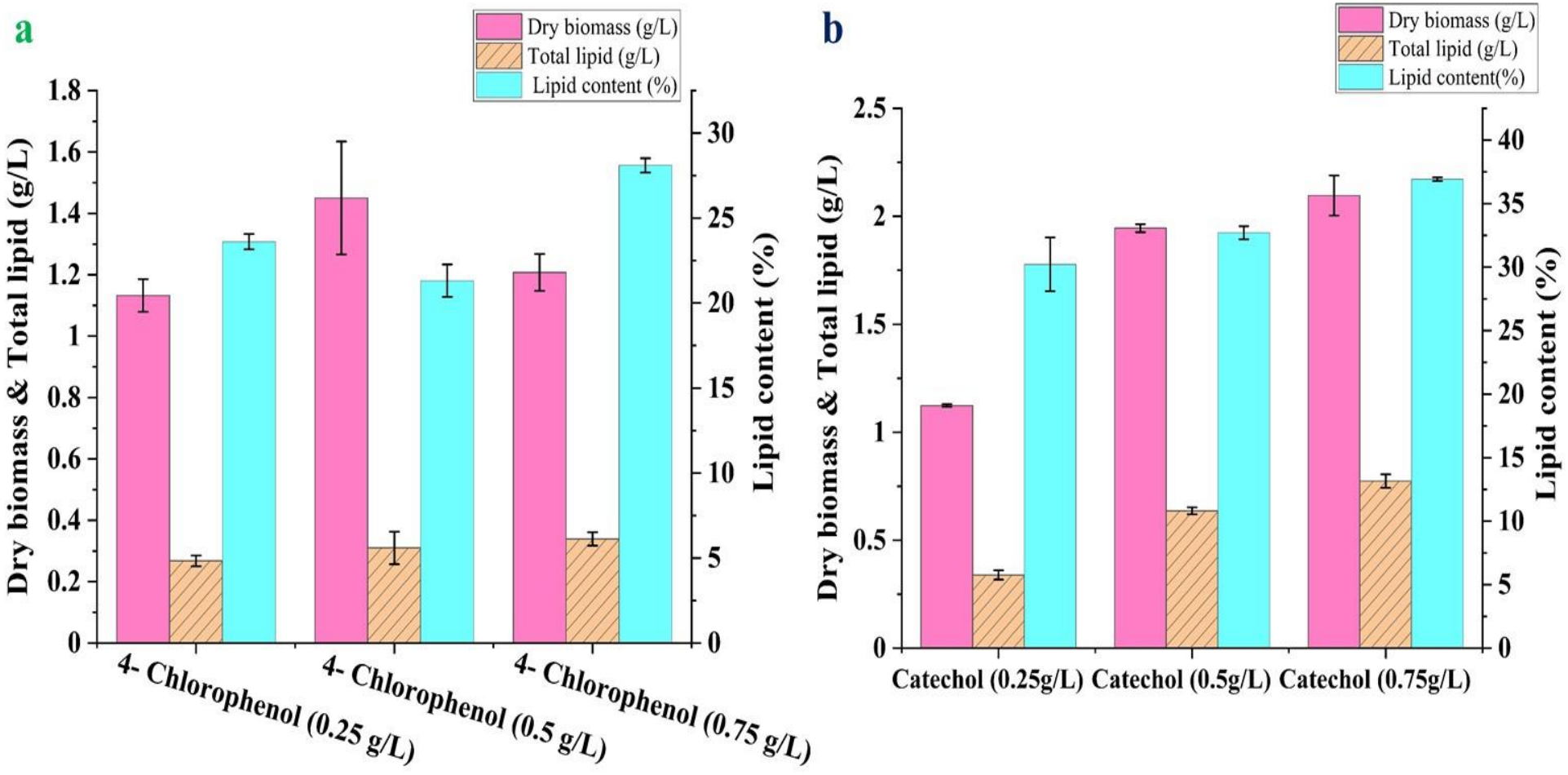


Maximum growth of *R toruloides* in different phenol derivative :

- **a:** 4-chlorophenol 0.75 g/L (concentration)
- **b:** catechol 1 g/L.

Fig 1 (a, and b): Growth profile of *R. toruloides* 9564^{T} in MSM media containing 4-chlorophenol, and catechol

Dry biomass (g/L), total lipid (g/L) and lipid content (% w/w)

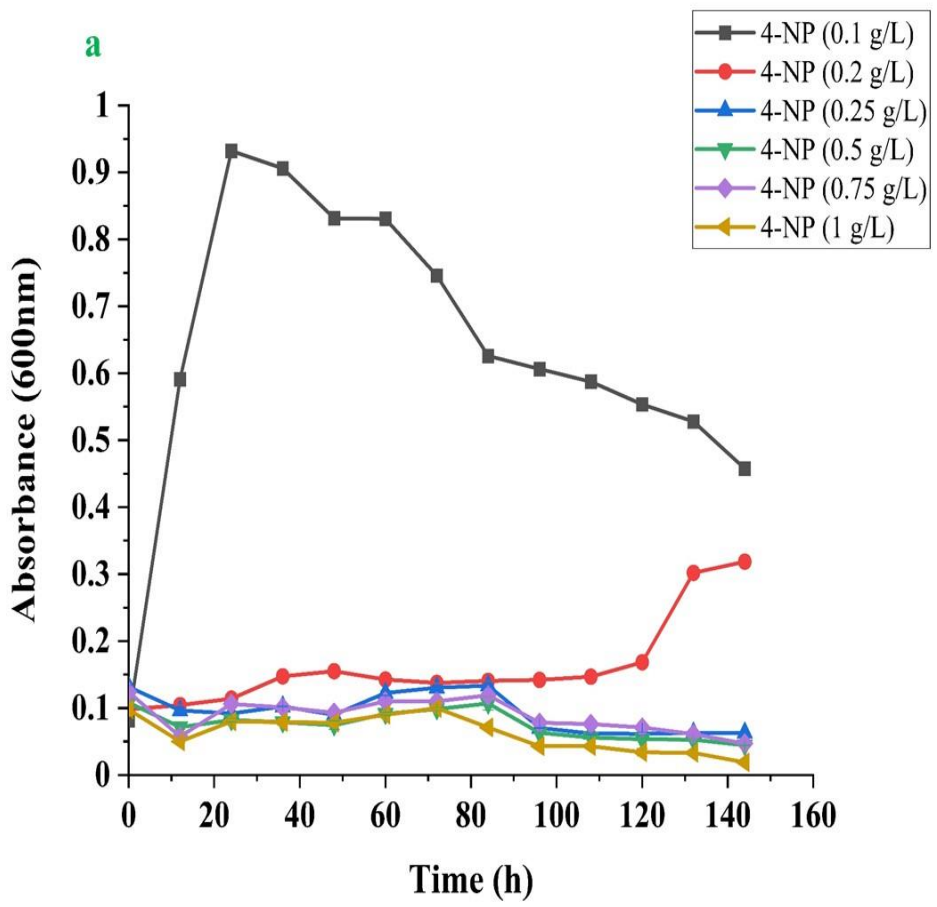


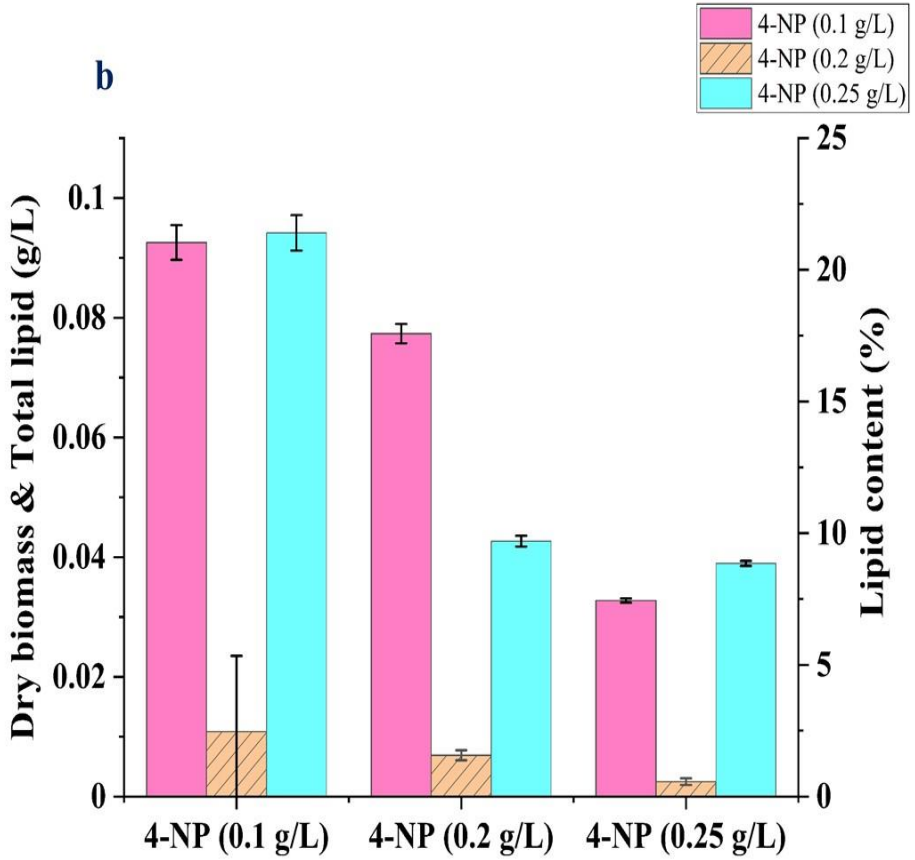
a : Dry biomass (1.2075 g/L) , Total lipid (0.3395 g/L) and Lipid content (28.12%)

b : Dry biomass (2.0965 g/L) , Total lipid (0.774 g/L) and Lipid content (36.92%)

Fig 2 (a and b): The graph shows dry cell biomass production (g/L), lipid yield (g/L), and lipid content (%; wt./wt.) of *R. toruloides* 9564^T in MSM with different concentrations of 4- chlorophenol and catechol.

4-nitrophenol degradation and lipid production



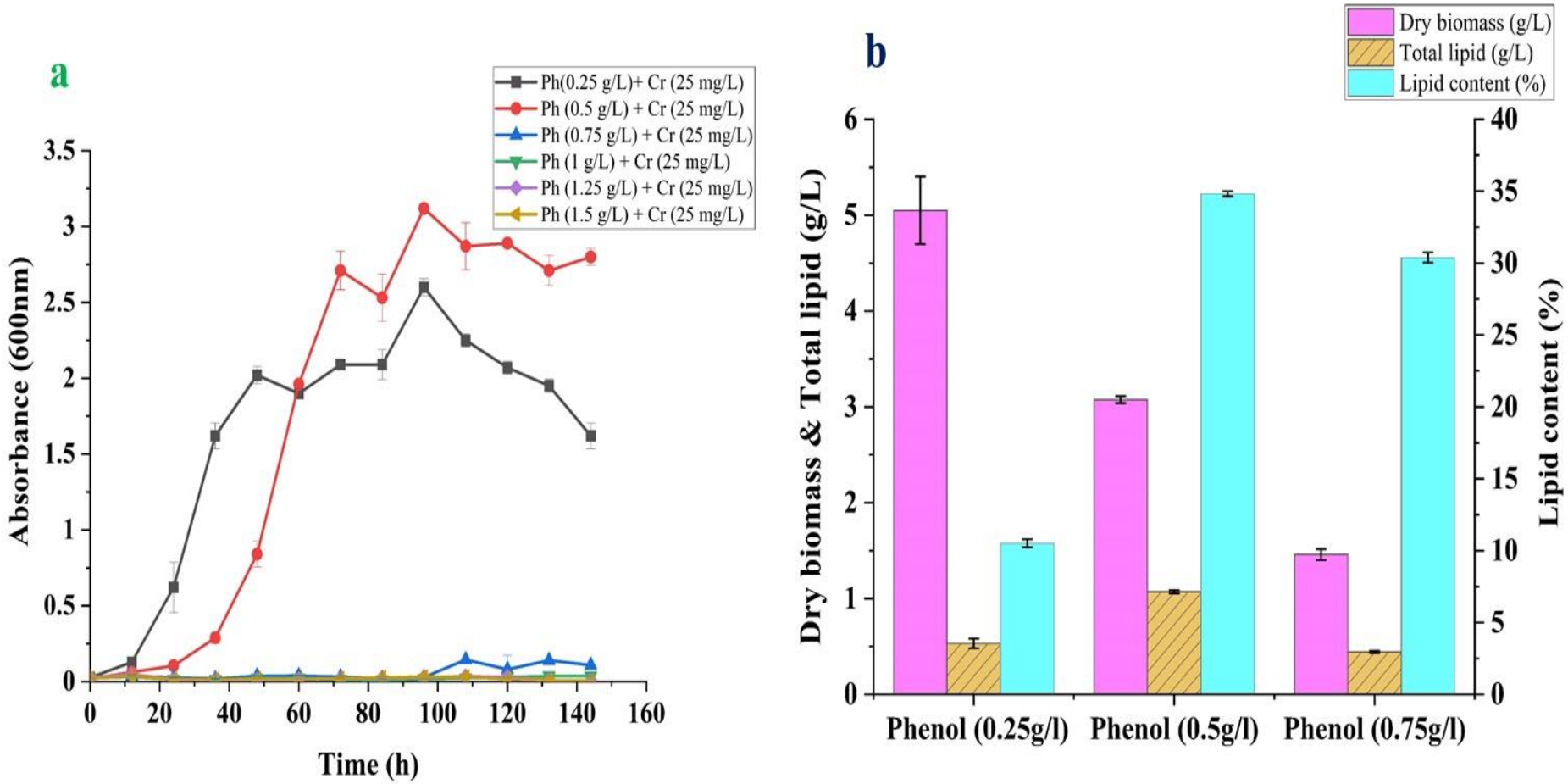


a: Maximum growth of *R toruloides* in 4-NP upto 0.1 g/L and log phase at 36h

b : Dry biomass (0.0925 g/L) , Total lipid (0.01084 g/L) and Lipid content (21.40%)

Fig 3 (a and b) : Growth profile and lipid extraction of *R. toruloides* 9564^{T} in MSM media containing 4-nitrophenol.

Effect of Chromium on phenol degradation and lipid extraction



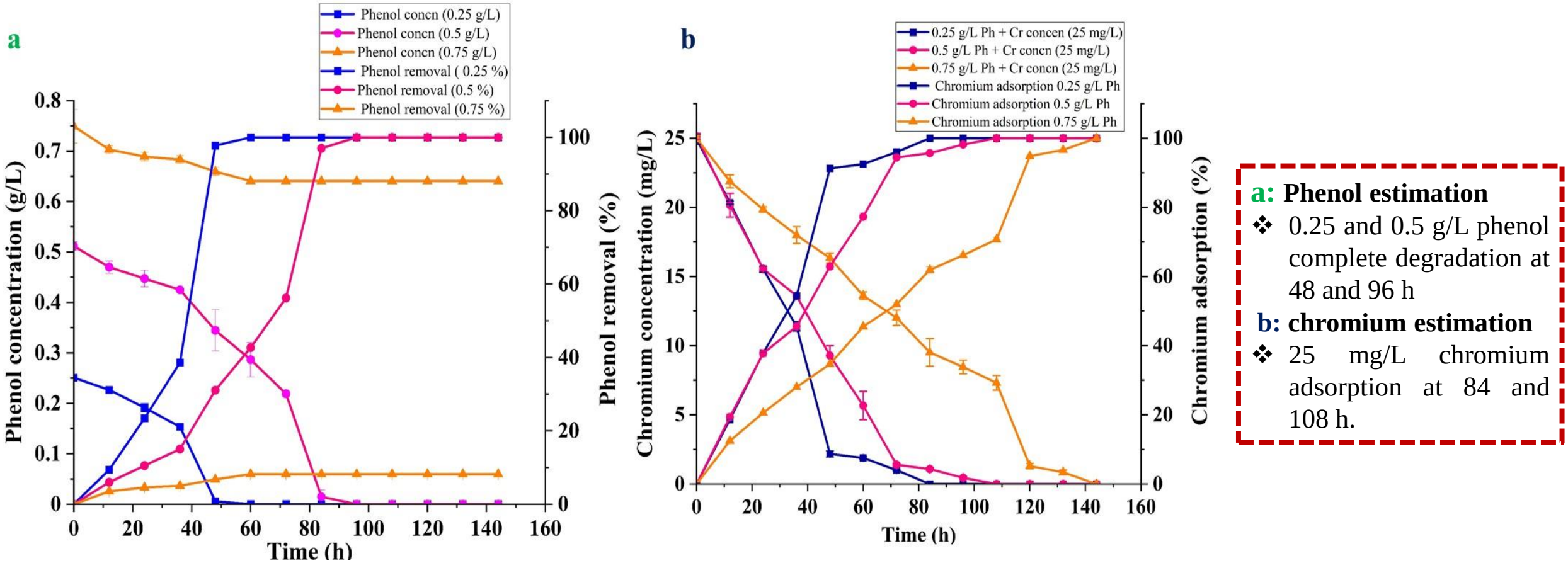
a: Maximum growth of *R toruloides*

- 0.25 g/L phenol and 25 mg/L: log phase 96h
- 0.5 g/L phenol and 25 mg/L chromium: log phase 108h

b : Dry biomass (5.05 g/L) , Total lipid (1.0705 g/L) and Lipid content (34.81%)

Fig 4 (a and b) : Growth profile and lipid extraction of *R. toruloides* 9564^{T} in MSM media containing phenol and chromium

Phenol degradation and chromium adsorption



a: Phenol estimation

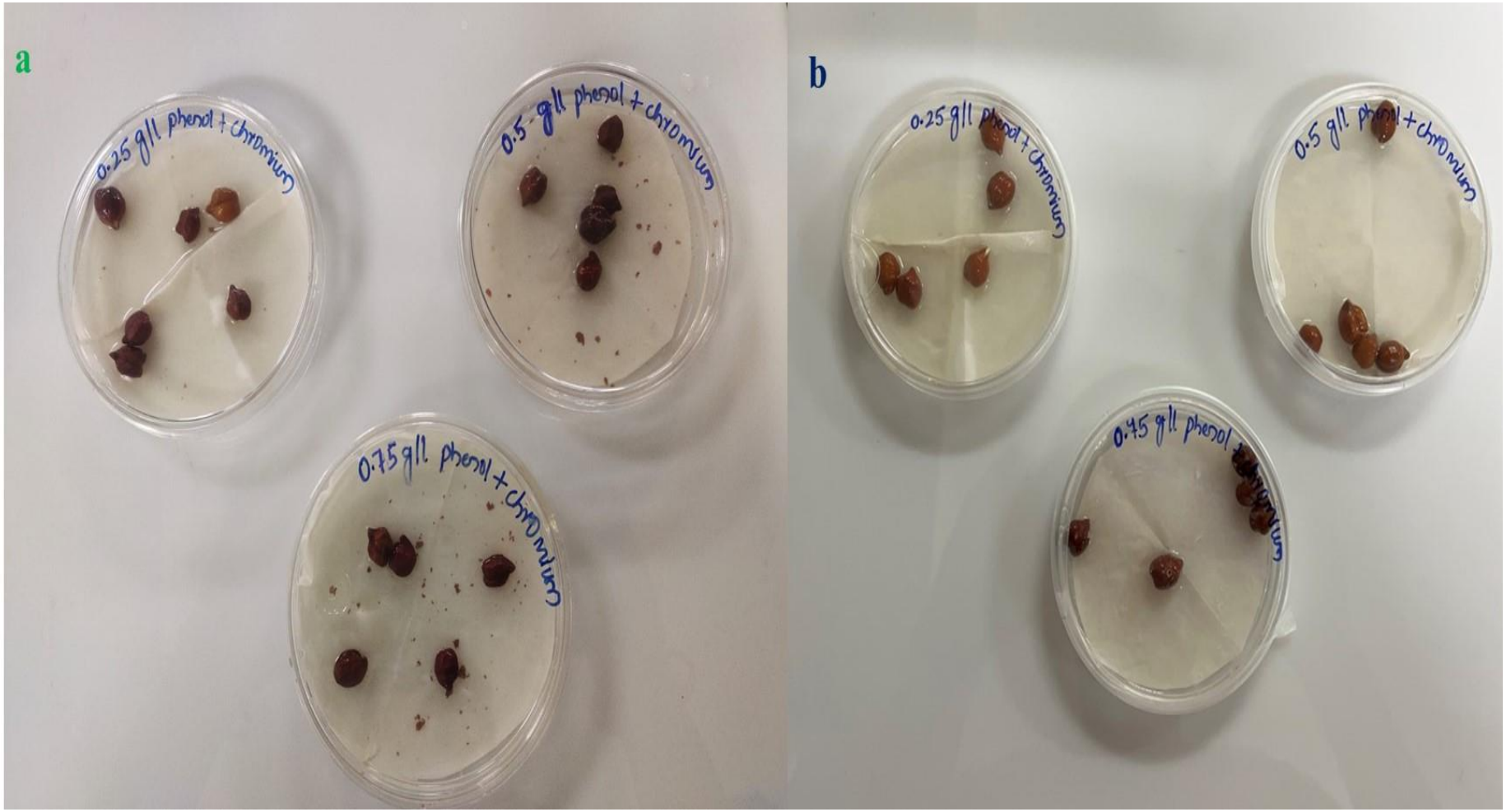
- 0.25 and 0.5 g/L phenol complete degradation at 48 and 96 h

b: chromium estimation

- 25 mg/L chromium adsorption at 84 and 108 h.

Fig 5 (a and b) : Phenol degradation and chromium adsorption by *R. toruloides* 9564^{T} in MSM media containing phenol and chromium

PHYTOTOXICITY

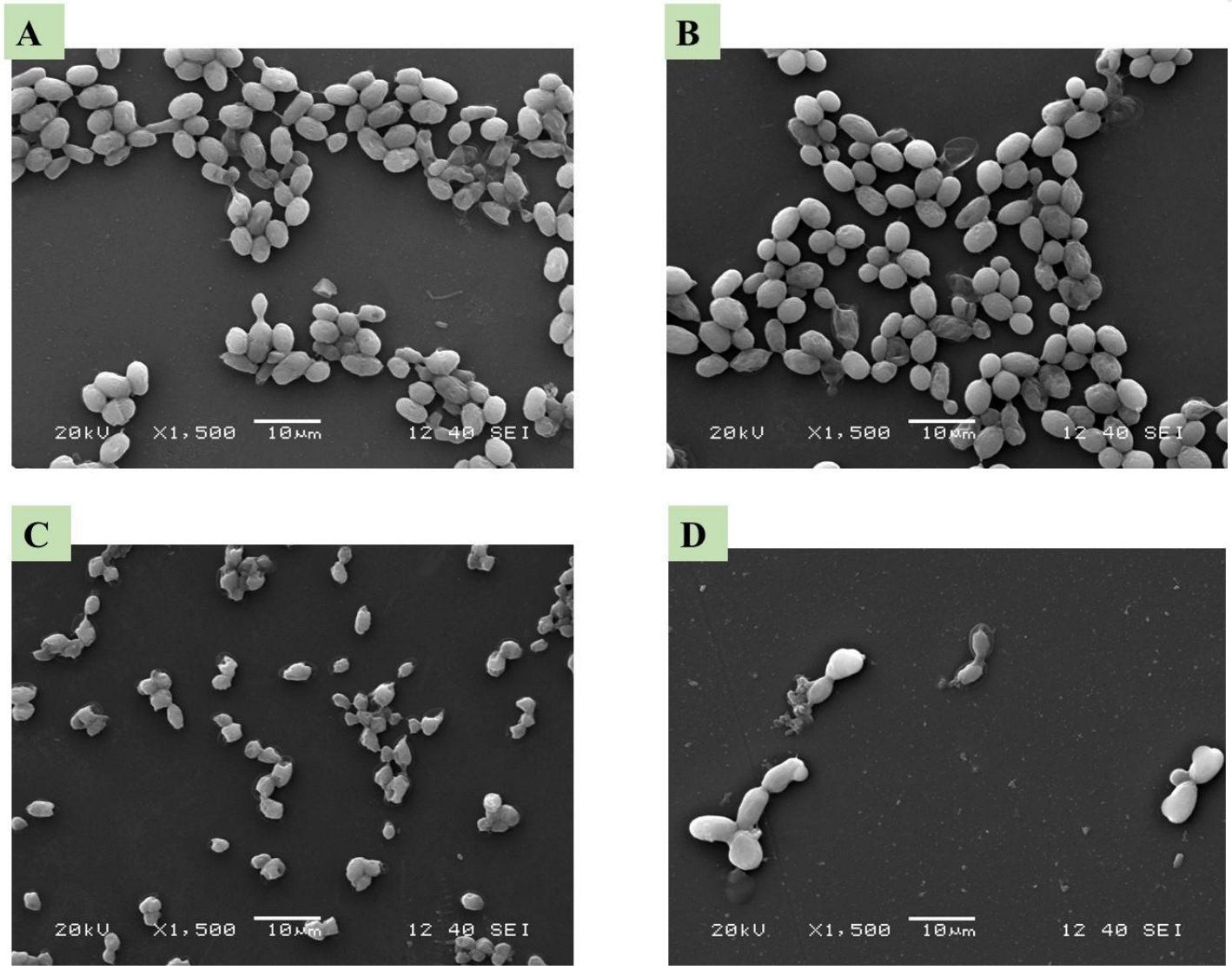


Phytotoxicity (*Cicer arietinum*):

- ❖ germination started in 0.25 and 0.5 g/L phenol and 25 mg/L chromium.
- ❖ No germination was shown in 0.75 g/L phenol and 25 mg/L chromium

Fig 6: Seed germination in phenol and chromium degraded sample.

CELL MORPHOLOGY



Cell morphology analysis by SEM

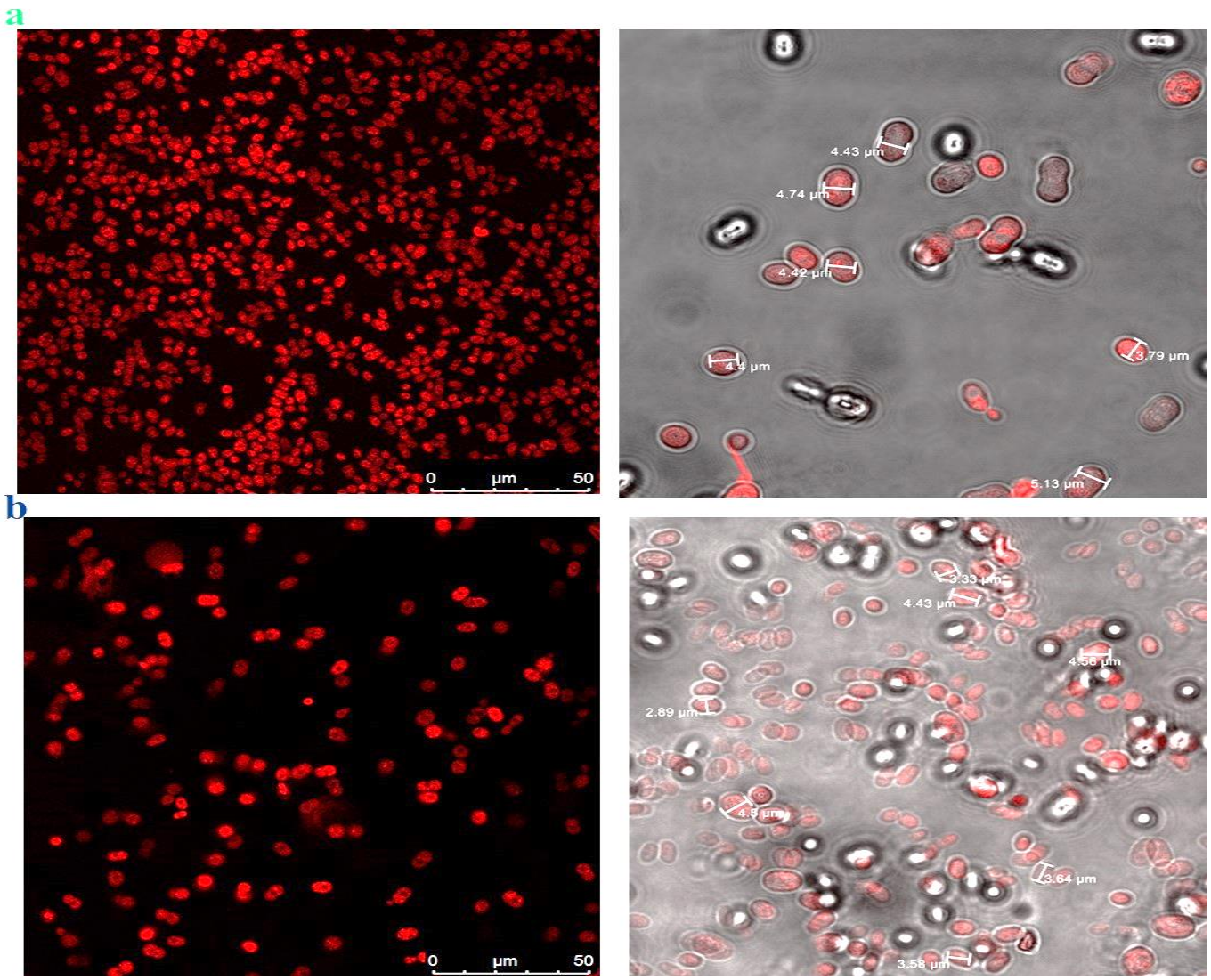
- A: *R. toruloides* culture in YEPD media.
- B: *R. toruloides* culture in MSM media.

Cell morphology analysis by SEM

- C: *R. toruloides* culture in MSM+Phenol media.
- D: *R. toruloides* culture in MSM+Phenol+Chromium media.

Fig 7: *R. toruloides* morphology analysis by SEM

Cell size and Lipid Droplets



Cell Size and Lipid droplet:

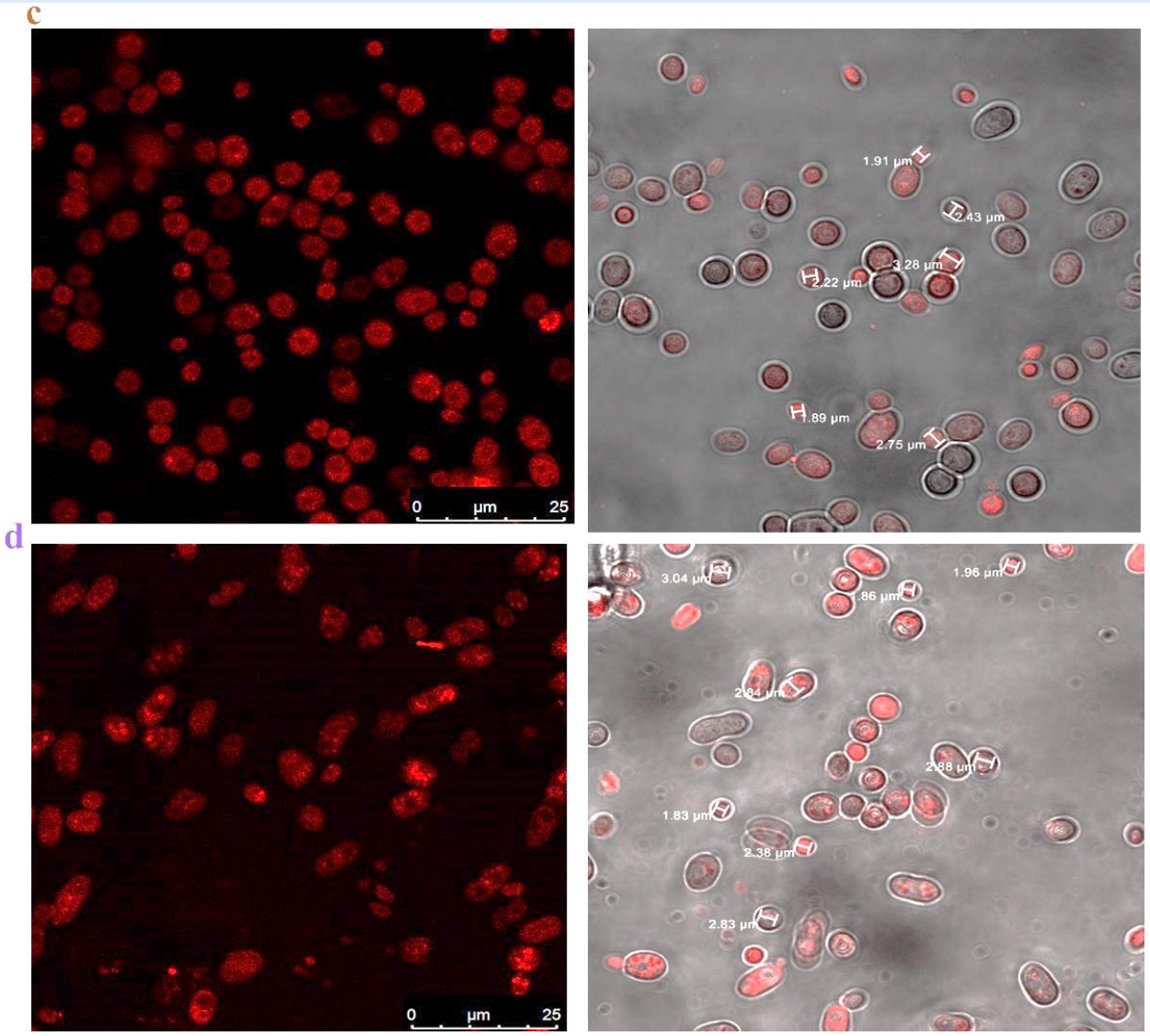
- A: cell size range between 3.5 to 5.2

Cell Size and Lipid droplet:

- B: cell size range between 3.5 to 4.6

Fig 8 (a and b) : Represent cell size and lipid droplets of *R. toruloides* in different media.

Cell size and Lipid Droplets



Cell Size and Lipid droplet:

- C: cell size range between 1.8 to 3.3

Cell Size and Lipid droplet:

- D: cell size range between 1.8 to 3.05

Fig 8 (c and d): Represent cell size and lipid droplets of *R. toruloides* in different media.

CONCLUSIONS

- The present study confirmed that *R. toruloides* 9564^{T} has the capacity to degrade **catechol, 4-chlorophenol** and **4-nitrophenol** (1 g/L, 0.75 g/L and 0. g/L).
- It can also survive in MSM media containing phenol and chromium upto **0.5 g/L phenol** and **25 mg/L chromium.**
- After degradation the phytotoxicity test confirm that upto 0.5 g/L phenol and 25 mg/L chromium combination are less toxic.
- Cell size in 4 different media also confirm the toxic level of phenol and chromium because in case of both the combination cell size are very low.


Thank You!