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RESEARCH ARTICLE

Biological recovery of phosphorus (BioP-Rec) from wastewater streams using brewer's yeast on pilot-scale

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Abstract

Most recent advances for phosphorus (P) recovery using brewery yeast on laboratory scale were used to scale up to a pilot-scale process (BioP-Rec module) and applied in a full-scale wastewater treatment plant (WWTP). A P balance was established for WWTP Markranstädt according to two thresholds: (1) the economic feasibility threshold for P recovery of 0.05 kg/m^3 of free P, and (2) the German Sewage Sludge Ordinance (GSSO) threshold, which demands that all WWTPs with a P content in dry matter (DM) of biosolids of 20 gP/kg$_{DM}$ or higher in the coming years must perform mandatory P recovery. In terms of defined thresholds, return and excess sludges were identified as the most feasible WWTP process streams for P recovery. In a 1 m^3 BioP-Rec module a 3 stage process was established. From the P-rich water-phase of the return sludge produced in stage 1, which contained 0.051 kg/m^3 of free P, 77.56% was taken up by P-depleted brewer's yeast *Saccharomyces pastorianus* in 3 h in stage 2. In stage 3, the yeast was concentrated in 1 h to produce yeast sludge as a fertilizer product. We demonstrated a novel pilot-scale process for the production of bio-based P-rich fertilizer.

KEYWORDS

biological phosphorus recovery, brewery yeast, pilot scale wastewater phosphorus recovery, *Saccharomyces pastorianus*

1 | INTRODUCTION

The recovery of critical resources from waste and their reintegration into production streams is a current priority. Phosphorus (P) as an EU critical raw material [1] is essential for living organisms and is primarily acquired from P rock ore [2, 3] which contains significant amounts of heavy metals [2, 4, 5]. Most P is used in agriculture as a fertilizer [6]. Without P, global food production would be impaired, and therefore, P should be recycled wherever possible, for example, from wastewater. In wastewater, human urine and feces account for about 3 Mt P/a [7, 8] and if recovered could potentially cover 20% (19.1 Mt/a) of the P needed for agriculture worldwide [8].

P recovery technologies from wastewater are currently focusing on full-scale chemical P recovery [9, 10] with struvite as a product (P recovery up to 95%) or P recovery

Abbreviations: BioP-Rec, biological P recovery process; DM, dry matter; FM, fresh matter; GSSO, German Sewage Sludge Ordinance; IBC, intermediate bulk container; P, phosphorus; WWTP, wastewater treatment plant.

from sludge ash after mono-incineration [11–13]. However, chemical recovery to struvite needs the addition of magnesium which is also an EU level critical resource [1], or high amounts of acids and bases for P recovery from sludge ash which is not environmentally friendly [14, 15]. Contrastingly, biological P recovery technologies are still mainly on a laboratory scale or on pilot scale [16, 17]. Here, we have recognized a research gap, and to support biological P recovery application on a larger scale, we demonstrate a novel biological P recovery process (BioP-Rec) from wastewater using brewer's yeast. Brewer's yeast can take up up to 28% of poly-P on a dry matter (DM) cell basis [18] and on a laboratory scale has been well optimized [18–20], which support our scale-up. Yeast is regarded as a safe microorganism for human application (GRAS) with around 15–18 t of surplus brewery yeast per 10,000 hectoliter of beer fermented available [21], qualifying it for fertilizer application [22].

The European Union and national legislative initiatives to support P recovery from wastewater are currently having a major impact on the search for solutions [1, 5, 23]. For instance, the German Sewage Sludge Ordinance (GSSO) demands that all wastewater treatment plants (WWTPs) above 50,000 population equivalents (approx. 500 WWTPs that treat 2/3 of Germany's wastewater) will need to compulsory recover the P if their biosolids have a P content in DM higher than 20 gP/kg_{DM} [24]. Starting from 2029 this will first impact the WWTPs with more than 100,000 population equivalents. Furthermore, total P above 0.05 kg/m^3 is considered feasible for economical P recovery [25]. Therefore, the objectives of this study were: (i) to define, suitable for P recovery, WWTP process streams with high P concentrations and P content in DM above 20 gP/kg_{DM}, (ii) to create sludge-free water-phases from sludge with free P (ortho-P) concentrations above 0.05 kg/m^3 as only free P can be taken up by yeast (or plants), (iii) to reduce the P content in DM in sludge streams below 20 gP/kg_{DM} to comply with the GSSO, and (iv) to test if brewer's yeast is able to accumulate free P to produce a P-rich fertilizer. A three-stage pilot process (BioP-Rec module) was tested for the applicability and efficiency of biological P recovery and the production of a biologically based fertilizer.

PRACTICAL APPLICATION

This work is meant to support the transfer of an environmentally friendly, biological-based phosphorus (P) recovery technology using wastewater treatment plant (WWTP) process streams. We demonstrate a 1 m^3 pilot-scale biological P recovery process (BioP-Rec) that comprises three stages to produce P-rich environmentally friendly fertilizer by using brewer's yeast – ready-to-use for application in agriculture. The constraint for economically feasible P concentrations for recovery from WWTP process streams was respected, as was the German Sewage Sludge Ordinance law regulation. The developed pilot plant is a first step toward achieving biological P recovery from wastewater streams and has the potential to be used on larger scales.

2 | MATERIALS AND METHODS

2.1 | WWTP P balance

In the WWTP Markranstädt (51 °18′13.5″N, 12 °12′173″N, Germany), 14 sampling campaigns at 21 sampling points, representing different process streams, were performed in a time period of 3 months as described in Supplementary Information (SI) 1. The process streams have been numbered according to their position in the WWTP scheme in Figure S1.1.

2.2 | BioP-Rec module process setup and experimental design

The BioP-Rec module (Figure 1, technical details in SI 2) was designed to use 12.return sludge as means to recover free P. The feasibility of using 20.biosolids was also tested (SI 3.3). The BioP-Rec module has three stages (Figure 1). Stage 1 was used to produce a free P-rich water-phase from 12.return sludge. Stage 2 supported yeast washing and growth on P-depleted medium as a pre-treatment step (2a), followed by the uptake of free P by this yeast (2b) by mixing them with the P-rich water-phase produced in stage 1. Stage 3 was a big bag filter that concentrated the yeast to produce P-rich yeast fertilizer as a product. Table 1 gives an overview of the experiments performed for the study with 12.return sludge.

2.2.1 | BioP-Rec stage 1 – Free P enriched water-phase production

The aim of stage 1 was to create a free P-rich water-phase from 12.return sludge with at least 0.05 kg/m^3 of free P and lower the P content in sludge DM to below 20 gP/kg_{DM}. For free P release, an intermediate bulk container (IBC) tank was filled up to 1000 L with return sludge and kept

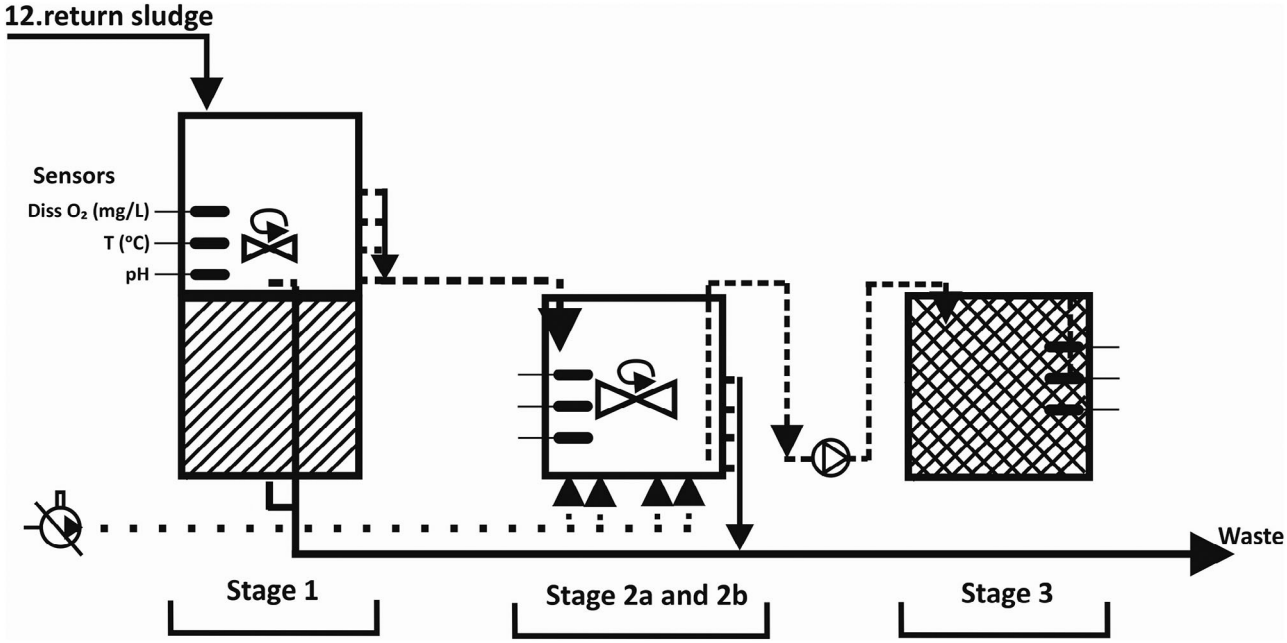

FIGURE 1 Scheme of the BioP-Rec module stages of a pilot scale of 1 m^3: stage 1 anaerobic free P release from 12.return sludge to produce a free P-rich water-phase; stage 2a yeast pre-treatment followed by stage 2b, aerobic free P uptake using stage 1 P-rich water-phase and stage 2a yeast; stage 3 filtration to concentrate yeast sludge as a product (details SI 2).

under anaerobic conditions. Free P release was tested on return sludge alone (positive control, experiment 3) and supported by the addition of 1.5 L of citric acid (experiment 1) and 1.85 L of citric acid (experiment 2). A stirrer (SI 2) was used at 1500 rpm to homogenize the sludge for 1 h (experiment 1) and 3 h (experiments 2 and 3). To separate biomass, return sludge was sedimented by gravitational forces in experiment 2 and 3 for 24 and 72 h, respectively, and with the addition of 600 mL of flocculant (Reiflock RF 600, Reiflock Abwassertechnik GmbH, Baden-Baden, Germany, Table S2.1) in experiment 1 after 1 h of free P release, for the next 2 h. The resulting upper liquid phase from experiment 1 was low in sludge particles and microorganisms and was used as P-rich water-phase for stage 2b after resting overnight in stage 1. Experimental details and chemicals are shown in SI 2 and SI 3. Free P release from 20.biosolids, methods and results are shown in SI 3.3.

Free P maintenance control for stage 1 water-phase

To ensure that there are no competing biological or chemical free P removal mechanisms, two negative controls were introduced. Free P-rich water-phase (400 L, 0.065 kg/m^3 of free P) from experiment 1 was transferred from stage 1 to stage 2b within 30 min and was treated with 200 L/min aeration and 50 rpm mixing for free P maintenance. In addition, an artificially prepared free P-rich water-phase of 0.065 kg/m^3 (KH_2PO_4) in 500 L of WWTP effluent was treated in the same way. Both experiments did not show loss of free P concentration (Table S3.2).

2.2.2 | BioP-Rec stage 2a – Yeast pre-treatment

The *Saccharomyces pastorianus* lager beer strain, acquired from an active brewery, was used for P uptake experiments. The strain originated from cylindro-conical storage tanks from up to four serial repitchings and was sampled and transported in 20 L containers. After 48 h storage at 4°C, the yeast sludge was separated from the free P-rich solution (1–2 L) using a suction gun (SI 4). Following, 10.5 kg of drained yeast sludge was transferred to stage 2a, where leftover extracellular P was removed in two sequential washing steps using 400 L of effluent water, mixed at 50 rpm for 10 min, settled, and drained for 15 min to leave 80 L of yeast and water. The settled yeast was then flooded with 400 L of effluent water amended with P-depleted medium (Table S2.1, stage 2a). The yeast was grown for 48 h to deplete the intracellular P pools and produce additional biomass. Growth medium was repeatedly neutralized using 30% KOH to keep the pH between 5 and 6 (SI 4). After 48 h the proliferated yeast was again washed with 400 L of effluent water, settled, and drained to leave 80 L of yeast and water. This yeast was used in stage 2b.

TABLE 1 Summary of key experimental details for stage 1 – release of free P release from return sludge to create a free P-rich water-phase; stage 2a – yeast pre-treatment by draining, washing, and P-depleted growth; stage 2b – P uptake by P-starved yeast, and stage 3 – product formation by yeast filtration.

Stage 1 – 12.return sludge free P release						
Experiment /n-repetition	**Working volume**	**Mixing**	**Citric acid**	**Flocculant**	**Biomass separation**	**Step duration**
1 ($n = 7$)	1000 L	First 1 h 1500 rpm	1.5 L	600 mL	Sedimentation	3 h
2 ($n = 2$)	1000 L	First 3 h 1500 rpm	1.85 L			24 h
3 ($n = 4$)	800 L					72 h

Stage 2a – yeast pre-treatment – draining, washing, starvation					
Experiment /n-repetition	**Treatment step**	**Working volume**	**Mixing**	**Chemicals**	**Step duration**
Stage 2a – yeast pre-treatment ($n = 3$)	Liquid phase draining	2 × 7.5 kg of brewery yeast			30 min
	Yeast washing	2 × 400 L	50 rpm		90 min
	Yeast starvation	400 L	50 rpm	P-absent growth medium composition as shown in SI 2	48 h
	Yeast washing	400 L	50 rpm		45 min

Stage 2b – yeast free P uptake								
Experiment/n-repetition	**Used P-rich water-phase**	**Used yeast**	**Working volume**	**Mixing**	**Aeration**	**Sucrose**	**Sunflower oil**	**Step duration**
Stage 2b ($n = 3$)	P-rich water-phase from experiment 1	Yeast from 2a	400 L	50 rpm	200 L/min	25 kg/m^3	30 mL	3 h

Stage 3 – yeast filtration to get the product					
Experiment/n-repetition	**Used water- phase and yeast**	**Working volume**	**Pumping**	**Yeast separation**	**Step duration**
Stage 3 ($n = 3$)	Product from experiment 2b	400 L	2000 L/h	Filtration	1 h

Specific details can be found in SI 2.

2.2.3 | BioP-Rec stage 2b – Yeast free P uptake and accumulation

For stage 2b free P recovery, P-depleted yeast (on average 4.239 kg/m^3 yeast DM) from stage 2a was flooded with 400 L free P-rich water-phase created in experiment 1 from stage 1. A 25 kg/m^3 of sucrose was added as a carbon source, together with 30 mL of sunflower oil to prevent foaming (Table S2.1, stage 2b). The process was run for 3 h under aerobic conditions at 200 L/min of aeration and 50 rpm of mixing.

2.2.4 | BioP-Rec stage 3 product generation

In stage 3, a big bag filter was used to filter the P-enriched yeast from the liquid, thus reducing the volume for later use as fertilizer. The yeast was pumped from stage 2b to stage 3 with 2000 L/h (SI 2) and separated from the liquid within 1 h.

2.2.5 | BioP-Rec module sensors

In all stages, as a standard, dissolved O_2 (mg/L), pH, and T (°C) were measured with sensors (SI 2), operated, and calibrated according to manufacturer instructions.

2.3 | Analyses and calculations

2.3.1 | Determination of total P, free P, and bound P

The analysis of total P [kg/m^3], free P [kg/m^3], and bound P calculation [kg/m^3] for the P balance of WWTP Markranstädt of all process streams and analysis of the P content in DM of all process streams and yeast in the BioP-Rec module were done according to Vucic et al. (2021) [26]. Also in this study, the terms free P, total P, and bound P are defined as free P dissolved in water (ortho-P = free P), as total P of a sample, or as difference between total P and free P, which includes P bound in microorganisms and P precipitated, respectively. The determination of the same values for 20.biosolids is provided in SI 3.

Measured free and total ortho-P were calculated as elemental free P or total P with a conversion factor of 3.07 for PO_4^{3-} to P [27]. For routine analysis of free P and total P, the Nanocolor Phosphate 15 kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany) and the Hach LCK 350, LCK 349, LCK 348 kit (Hach Lange GmbH, Düsseldorf, Germany) with the D 500 spectrometer and the HACH DR 3900 spectrometer were used. The comparability between the Nanocolor and Hach P determination kits was demonstrated by calibration curves (SI 5). In addition, free P was measured with a method modified from Taussky and Shorr (1952) [28] to mitigate costs (SI 6). All measurements with the Taussky method were done in parallel, and as a single or parallel when using Nanocolor or Hach cuvette tests. P content in yeast was measured by Nanocolor cuvette test and ICP-OES analyses (SI 8).

2.3.2 | Fresh matter (FM) and dry matter (DM) measurement

FM and DM concentrations [kg/m^3] were measured using two different methods for WWTP low biomass streams and high biomass streams (SI 7). For both types of streams standardized daily measurement procedures were used. Low biomass streams were 1.inflow municipal; 2.inflow industrial, 3, 4, 5.grit chambers, 19.centrate, and 18.distribution shaft. Here, FM was determined by using 120 mL to 600 mL of process streams to concentrate approximately 1 g of FM with centrifugation steps at 3200 *g*, 10 min, 4°C using 50 mL Falcon tubes filled with a 40 mL sample. The resulting pellet was transferred into 2 mL Eppendorf tubes and centrifuged at 6000 *g*, 10 min, 4°C. The supernatant was discarded, and FM was measured. High biomass streams were 6, 7, 11.nitrification basin, 8, 9, 10.denitrification basins, 12, 13, 14.return sludge, and 15, 16, 17.excess sludge. FM concentration was determined by using 2 mL of process streams in 2 mL Eppendorf tubes or 40 mL of process streams in 50 mL Falcon tubes. The tubes were centrifuged at 3200 *g*, 10 min, 4°C and, after removal of the supernatant, at 6000 *g*, 10 min, 4°C. For both types of streams the tubes were dried for 3 days at 50°C and DM was measured. All measurements were done in triplicates. For the low biomass streams FM-DM conversion factors were determined (Table S7.1). Controls and devices are presented in SI 7.1. In addition, a comparison between Hach TSS values obtained from the WWTP and our values for high biomass streams can be found in Table S7.2.

Furthermore, FM and DM concentrations [kg/m^3] were determined for yeast sludge (SI 7.2). The yeast specific growth rate as a change of yeast DM concentration over 48 h was calculated for stage 2a. In the BioP-Rec module stage 2b, the average yeast DM concentration was used for calculation of P content in DM.

2.3.3 | Calculation of P content in DM

The P content in DM in the WWTP P balance and for the BioP-Rec module was measured and calculated as described in SI 6–8. The BioP-Rec module yeast P content

in DM was additionally controlled by ICP-OES as shown in SI 8. The accumulation of P in yeast cells was calculated as a difference between the start and the end of the P content in DM in BioP-Rec stages 2a and 2b.

2.3.4 | Calculation of WWTP Markranstädt P balance

WWTP Markranstädt P balance was calculated according to Vučić et al. (2021) [26]. An adaptation of the calculation pipeline due to the differences in WWTP design is shown in SI 1.

3 | RESULTS

3.1 | WWTP Markranstädt P balance

WWTP Markanstädt P balance was constructed with the aim of finding P-rich streams with total P over 20 gP/kg_{DM} and above 0.05 kg/m^3 and, therefore, feasible for P recovery [25, 26]. The available P fractions for P recovery were free P found in water-phases and bound P found in solid phases of WWTP process streams, shown as a total P (SI 1). Out of 21 sampling points, 12, 13, 14.return sludge (0.295–0.301 kg/m^3, also excess sludge with the same properties), and 20.biosolids (0.269 kg/m^3) with total P above 0.05 kg/m^3, and a P content in DM (28.459–30.590 gP/kg_{DM}) above the 20 gP/kg_{DM} threshold of the GSSO were deemed feasible for P recovery. Being the most promising and the easiest to access, 12.return sludge was taken to create a free P-rich water-phase for the recovery of P using yeast. To ensure economic feasibility, we required a concentration of 0.05 kg/m^3 of free P in the P-rich water-phase, which differs from the same value for total P in ref. [25].

3.2 | BioP-Rec process stage 1 – Free P release from the return sludge

Stage 1 was designed to facilitate anaerobic free P release from wastewater sludges. In the return sludge of the WWTP Markranstädt, we found less than 1% of free P (0.001 kg/m^3, SI 1.3). Therefore, in stage 1, P bound in sludge solid phase needed to be released to the water-phase as free P in an amount of at least 0.05 kg/m^3. Without any treatment of the return sludge (control experiment 3, Figure 2A) 3 days were needed to release free P to reach 0.05 kg/m^3 under anaerobic conditions (SI 3.1). Therefore, citric acid was used to increase the release of free P from the solid phase of the sludge in experiment 1 at a pH of

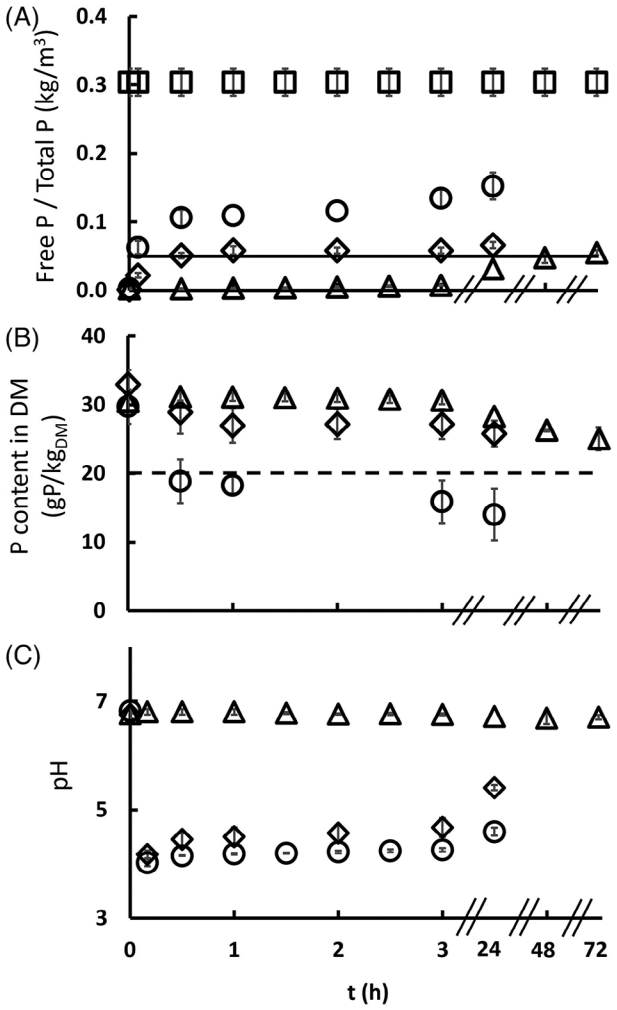

FIGURE 2 Release of free P from return sludge in BioP-Rec module stage 1. (A) □ total P average of experiments 1–3. The free P release in return sludge was tested under anaerobic conditions in △ experiment 3 (positive control), after addition of citric acid: ◇ 1.5 L (experiment 1), or ○ 1.85 L (experiment 2). 600 mL of flocculant was added after 1 h of free P release in experiment 1. The thick black line is the economic feasibility threshold of 0.05 kg/m^3. In the citric acid experiments, the black line was reached after a minimum of 30 min. (B) P content in DM during free P release decreased below 20 gP/kg_{DM} (dashed black horizontal black line is the GSSO threshold) in ○ experiment 2. (C) pH profile during free P release in stage 1. In ◇ experiment 1 – pH rose to 5.4 over 24 h, ○ experiment 2 – pH rose to 4.5 over 24 h. In △ control the pH was stable. In all experiments dissolved O_2 was 0 mg/L, and T stable (SI 3.1).

4.1 (1.5 L of citric acid per 1000 L of sludge) within 2 h and in experiment 2 at a pH of 4.0 (1.85 L of citric acid per 1000 L of sludge) within 24 h (Figure 2A,C). Free P reached 0.051 kg/m^3 (experiment 1) and 0.106 kg/m^3 (experiment 2) within 30 min thus complying with the economically feasible free P threshold. The release of free P was accelerated

133- and 277-fold, respectively, compared to experiment 3 with a duration of 3 days. In addition, two negative controls verified the maintenance of free P concentration in the water-phase for 3.5 h (free P-rich water-phase from experiment 1 and artificially prepared free P-rich water-phase, SI 3.2) to ensure that no competing mechanisms reduce the free P in the P-rich water-phase of stage 1. The negative controls were obtained from experiment 1 and from an experiment where artificial free P concentrations were applied (Table S3.2).

A water-phase rich in free P is required to contain only low amounts of microorganisms. For the duration of the free P release, in all experiments the return sludge biomass was allowed to settle to separate the upper water-phase from sludge. After 1 h of free P release, an iron-free flocculant was added to accelerate the sludge settling in experiment 1, resulting in 400 L of water-phase after 2 h ready-to-use for P recovery in stage 2b (SI 3.2, Figure S3.3). The flocculant did not cause a decrease of free P, creating a water-phase with a biomass concentration of only 2%–3% (SI 3.2). Without the flocculant, the separation of the sludge from the water-phase took at least 24 h (experiment 2). The water-phase further used for stage 2b was from experiment 1, after overnight resting in stage 1. In this time, the pH of the water-phase changed from initial pH 4.1 to 5.4 (Figure 2C) and free P is present in a $H_2PO_4^-$ bioavailable form [29].

The release of free P from return sludge lowered the P content in DM from 32.947 to 25.789 gP/kg_{DM} after 24 h in experiment 1. However, a higher citric acid content resulted in a lower P content in DM below the GSSO threshold of 20 gP/kg_{DM} (Figure 2B). In experiment 2, 18.844 gP/kg_{DM} was already reached within 30min.

To summarize, return sludge was the most impactful WWTP process stream and used in stage 1 to create a low-biomass, free P-rich water-phase within 3 h, with economically feasible free P concentrations, thereby producing sludges that are within the GSSO compliance.

3.3 | BioP-Rec process stage 2a – Yeast pre-treatment

Brewer's yeast had to be pre-treated before it could be used for P recovery because it was first suspended in a solution rich in free P coming from the brewing process and, second, full of intracellular poly-P. Both circumstances hinder a successful use of yeast as a means to take up the free P from the water-phase produced in stage 1. Therefore, the free P-rich solution of the yeast sludge must be removed, and the yeast cells must be freed from their intracellular poly-P reserves. These two processes were performed in stage 2a. The free P-rich solution (0.36 kg/m^3) from the yeast sludge containers was drained (1–2 L) with a suction gun (SI 4) to be directly available as a P-rich fertilizer. Following, the yeast was washed twice with 400 L effluent water to remove extracellular P from cell surfaces which led to only a slight decrease of total P and free P while the yeast P content in DM did not change (Figure S4.1A, B). The T was constant, and the pH and dissolved O_2 slightly decreased (Figure S4.1 C). Previous reports by Christ and Blank (2019) [18] and Qin et al. (2022) [20] demonstrated that yeast cells with low intracellular P content have a high capacity to accumulate free P and that these lower intracellular P concentrations can be achieved by culturing yeast cells on P-depleted medium. Therefore, to deplete the yeast of intracellular P, yeast was grown for 48 h in P-depleted medium to urge the use of intracellular reserves of poly-P for growth (Figure 3A–C). Indeed, we found that during growth, P content in DM of yeast dropped from 14.167 gP/kg_{DM} (ICP OES 15.859 gP/kg_{DM}) to 6.854 gP/kg_{DM} (ICP OES 6.239 gP/kg_{DM}) (2x decrease, ICP 2.5x) (Figure 3B, SI 8). Therefore, intracellular P was successfully lowered by growth under P starvation conditions. Biomass increased on average from 4.480 kg/m^3 of yeast DM to 9.353 kg/m^3 with a specific growth rate of 0.015 h^{-1} over 48 h. After growth, yeast was sedimented to 80 L, washed with 400 L of effluent water to remove growth medium, and sedimented again to 80 L within 45 min. This yeast was now ready for use in stage 2b.

3.4 | BioP-Rec process stage 2b – Uptake of free P by yeast

P-depleted yeast from stage 2a was used for P recovery from free P-rich water-phase from stage 1 (experiment 1, with remaining sludge DM biomass concentration of 0.246 kg/m^3) in stage 2b (Figure 3D–F). Within 3 h, the P content in yeast DM (Figure 3E, SI 8) increased from 6.854 gP/kg_{DM} (ICP OES 6.239 gP/kg_{DM}) to 21.150 gP/kg_{DM} (ICP OES 16.351 gP/kg_{DM}). This was a three-fold increase (ICP OES 2.6x) in P content in DM where starved yeast accumulated 14.736 gP/kg_{DM} (ICP OES 10.112 gP/kg_{DM}) with a free P recovery efficiency of 77.56% of the initial 0.0518 kg/m^3 free P of the water-phase. During the P recovery process, T was on average 22°C, dissolved O_2 around 7 mg/L and pH declined from pH 5.5 to 4 because of acidification from yeast metabolism (Figure 3F). The experiment was performed in three independent replicates. In stage 2b, we demonstrated at a pilot scale of 1 m^3 that yeast low in P content in DM is an effective means of P recovery from wastewater streams.

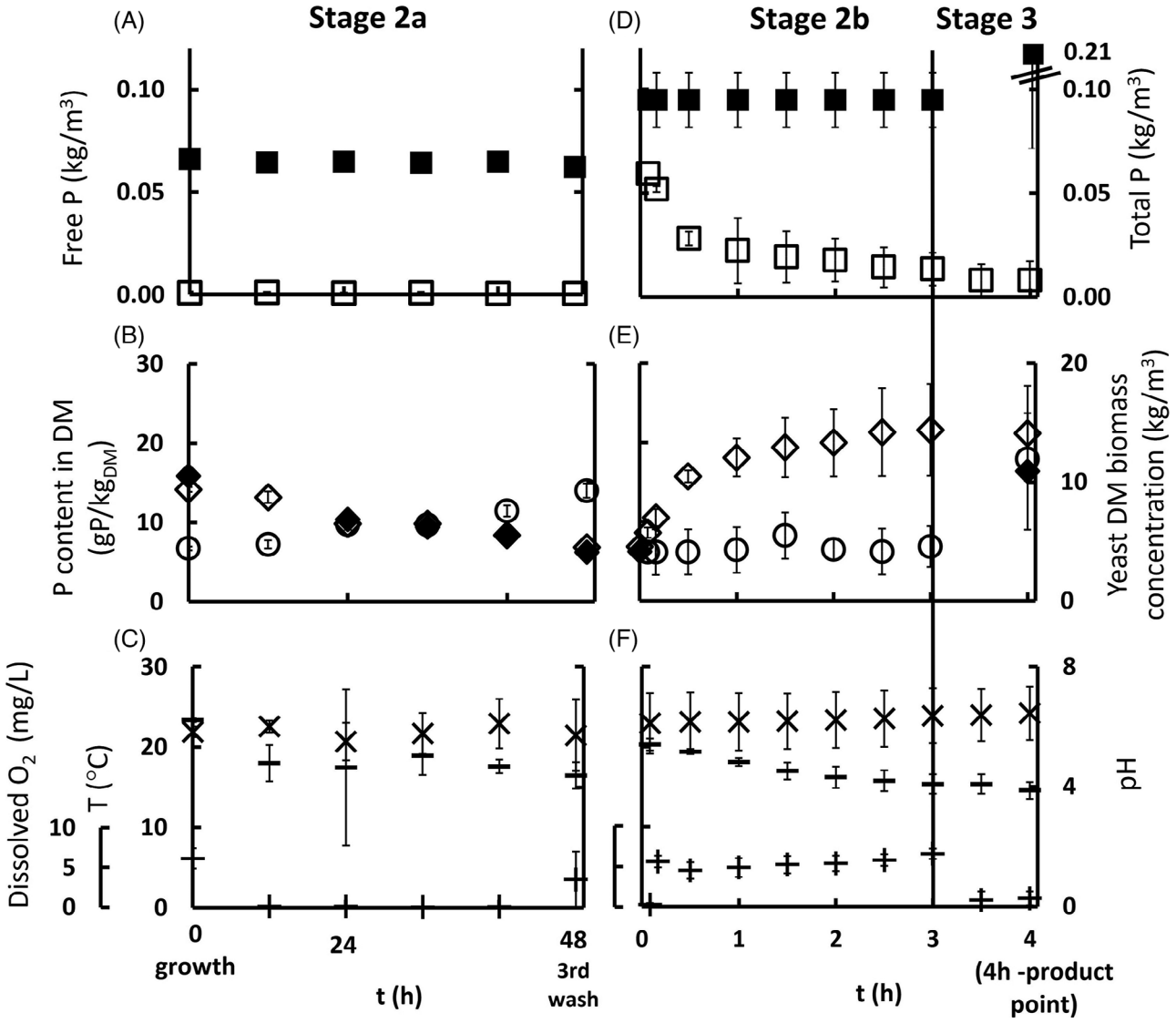

FIGURE 3 Uptake of free P (stage 2b) from free P-rich water-phase (stage 1, experiment 1) by yeast after yeast pre-treatment (stage 2a) and production of yeast fertilizer in stage 3. (A–C) Stage 2a – yeast pre-treatment by growth on P-depleted medium for 48 h. (D–F) Stage 2b – yeast P uptake, and stage 3 – generation of P-rich product. Parameters: (A, D) ☐ free P and ■ total P; (B, E) (SI 8): P content in DM measured by Nanocolor cuvette test ◇, P content in DM measured by ICP-OES ◆ and yeast DM ○. (C, F) + diss O_2, × T, and − pH. In (D–F) the vertical black line indicates yeast transfer and filtration into stage 3 where point 4 h shows the parameters of the product.

3.5 | BioP-Rec process stage 3 – Yeast filtration

In BioP-Rec stage 3, the fertilizer product was concentrated. Here, big bag filter (SI 2) was used to separate the yeast biomass from the liquid. A 400 L of yeast solution from stage 2b was pumped into stage 3, and after 1 h, about 5 L of the yeast product was harvested with an increase in DM from 4.239 kg/m^3 (st dev 1.877) to 11.933 kg/m^3 (st dev 7.153) or 2.7-fold (4 h point black square, Figure 3E). The variations in standard deviations of yeast DM were caused by ineffective yeast settling and filtration due to high T, where cells were lost due to the poor flocculation. There was no free P release from yeast cells (Figure 3D) and no loss of yeast P content in DM (Figure 3E) from 3 to 4 h. In stage 3, we have shown that yeast sedimentation and filtration are viable options for the separation of the yeast from the liquid solution to produce fertilizer.

4 | DISCUSSION

On a full-scale WWTP, technologies for P recovery exist and are based mainly on chemical P precipitation (see overview e.g., [22]). The costs of those technologies range from <1€/kg of P to more than 10€/kg of P recovered

[30–32]. In this study, we tested a pilot-scale approach based on biological P recovery which still cannot yet compete with established full-scale chemical-based P recovery technologies. But other biological-based P recovery technologies are still scarce [16, 17]. Therefore, considering the economical feasible free P concentrations and GSSO, and using the set of aims defined in the introduction, we discuss the status and feasibility of the BioR-Rec module, in which yeast was used to recovery P directly from return sludge of a WWTP. The specific problem in this study was that all streams (except biosolids) in the WWTP Markranstädt had free P values below 0.01 kgP/m^3. But even under these circumstances, the BioP-Rec module could be a first and valuable step to produce environmentally friendly fertilizer while reducing the P content in the biosolids below 20 gP/kg_{DM}. The BioP-Rec module was a three-stage process and was successfully applied to return sludge. In stage 1 free P was released in concentrations above 0.05 kg/m^3. In stage 2 brewery yeast *S. pastorianus* was starved of P by growth on medium depleted of P and used to take up free P from the P-rich water-phase produced in stage 1. The P-rich yeast fertilizer was formed and collected in stage 3. All stages are discussed in the following chapters.

4.1 | WWTP Markranstädt P balance

Before using the BioP-Rec module for P recovery, a P balance [26] identified the WWTP process streams that are richest in P and are therefore feasible for P recovery. The process stream chosen for P recovery in this study was return sludge fulfilling aim (i).

4.2 | BioP-Rec process stage 1 – P release from the return sludge

We fulfilled also aims (ii) and (iii) by creating P-rich water-phases with free P concentrations above 0.05 kg/m^3 and lowering P contents in DM below the GSSO threshold of 20 gP/kg_{DM}. The GSSO requires that beginning in January 2029, all WWTPs (>100,000 population equivalents) with P levels in DM biosolids of 20 gP/kgDM and greater [24] must recover P using available technologies.

An economically viable free P concentration of at least 0.05 kg/m^3 was achieved most rapidly when citric acid was added in stage 1 to assist P release to 0.106 kg/m^3 within 30 min (Figure 2A, experiment 2). Free P release was found to be lower with lower initial dosage of citric acid (experiment 1), similarly as reported for sludge acidification with acetic acid [33–35]. The reason for the faster free P release at lower pH is probably that low pH might lead to toxification from undissociated acid penetrating bacterial cell membranes [35]. Poly-P accumulating microorganisms might be forced to pump out excess acid which is an energy-intensive process. This energy is obtained from poly-P degradation processes causing high free P release [35]. The low pH caused by citric acids might also dissolve precipitated P from metal complexes [36]. In this study, we did not differentiate whether free P release was biologically or also partly chemically based in stage 1.

It has been reported that an efficient reduction of P content in DM of activated sludge also reduces P content in DM in biosolids [37, 38]. In our pilot plant the release of free P from sludge biomass after citric acid treatment caused a decrease of P content in DM below 20 gP/kg_{DM} in the return sludge (experiment 2) from 29.725 to 18.844 gP/kg_{DM}. Also Chaparro and Noguera (2003) found the P content in DM in biosolids lowered from 50 to 32 gP/kg_{DM} after free P release from activated sludge by support of volatile fatty acids [37]. Without using citric acid, the time required to produce a P-rich water-phase and lower P content in DM of sludge was 72 h (experiment 3). If a WWTP has sufficient space and basins to store return sludge long enough to release free P, this could be a way of releasing free P without chemicals.

We also tested the release of free P directly from dissolved biosolids (SI 3.3) in stage 1, but due to the lack in separating the free P-rich water-phase from the biosolids, we did not pursue this approach. Nevertheless, if a WWTP comprises a digester, the biosolids can be used as carbon- and energy source for the anaerobic microorganisms, and due to the anaerobic conditions, the contained biological bound P would be released into the centrate, resulting in a free P-rich water-phase [26]. This centrate could be fed directly to the yeast cells in stage 2b, avoiding stage 1. We should note that the same procedure can be done with return sludge if this material is used in a digester.

Further possibilities may exist to increase the efficiency of the BioP-Rec module stage 1 with additional technological upgrades. We have demonstrated that 400 L of free P-rich water-phase low in return sludge biomass, can be produced in 2 h using a flocculant, other flocculants might be even more effective. Although citric acid was required to release free P from return sludge in this study, the use of this chemical may not be necessary in WWTPs that have higher levels of free P in return sludge and meet the free P threshold (0.05 kg m^{-3}), for example, because a WWTP operation supports enhanced biological P removal (EBPR). Also, as mentioned above, streams such as centrate from digesters or centrate from biosolid dewatering steps with iron-free flocculants can easily reach this threshold, making the use of citric acid unnecessary.

Furthermore, additional dewatering and filtration technologies might improve biomass separation to speed up the process and avoid pathogens and other contaminants that may be present in sludge [39].

4.3 | BioP-Rec stage 2a, 2b – Yeast pre-treatment, free P uptake, and P accumulation

Fulfilling aim (iv) we showed that brewer's yeast *S. pastorianus* can be used for biological P recovery from return sludge. Yeast is known to accumulate free P in high concentrations (28% of poly-P on DM cell basis [18]), but they can also lose their intracellular P upon different types of shock, for example, high pH [40, 41]. In stage 2a, the draining and washing steps were non-invasive procedures that removed extracellular P but not intracellular P. Recent advances have shown that yeast initially cultivated on a P-deficient medium is able to accumulate free P rapidly and in high amounts. Christ and Blank (2019) reported for benchtop experiments an increase of P content in DM of P-starved yeast from 0.1 to 52 gP/kg_{DM} [18] and Qin et al. (2022) showed on the single cell level that initial growth on P-depleted medium led to an increased P-accumulation capacity [20]. Based on these findings, we initiated starvation by a growth step for yeast on P-depleted medium and used this yeast for P uptake from return sludge P-rich water-phase. In stage 2b, yeast P content in DM increased from 6.854 gP/kg_{DM} (ICP OES 6.239 gP/kg_{DM}) to 21.150 gP/kg_{DM} (ICP OES 16.351 gP/kg_{DM}) within 3 h. With this a successful transfer of P yeast uptake from laboratory to pilot-scale was achieved. The robustness of yeast was demonstrated by Powell and Diacetis (2007), who found no significant physiological differences in yeast after 135 generations [42]. Also, Bühligen et al. (2013, 2014) demonstrated for *S. pastorianus* its ability to maintain longevity due to the up regulation of autophagy, telomere silencing, and telomere protection genes with a stable rejuvenation rate during as many as 20 serial fermentation runs [43, 44]. Therefore, due to the current state of research on P uptake, robustness, and general availability, *S. pastorianus* seems like a good choice as a microorganism for P recovery. Other bench top protocols reported also high uptake rates, up to 38 gP/kg_{DM} [45] of P after starvation. It might be a future possibility to use engineered yeasts such as *Hansenula fabianii* and *H. anomala* which are able to accumulate huge amounts of P on the laboratory scale [46]. However, due to the unpredicted consequences for nature and human health [47], using genetically engineered strains is under strict regulation [48], and cannot be used as fertilizer in the open environment.

4.4 | BioP-Rec stage 3 – Yeast filtration to create the product

Yeast sedimentation and filtration using a big bag filter were the main approaches to concentrate the yeast in this study. Normally, *S. pastorianus* flocculates and settles at a temperature of 4°C without further intervention [49, 50]. However, due to the high external temperature (>20°C), the yeast did not settle well in our pilot plant and therefore had to be filtered. During this process the yeast did not lose accumulated intracellular P. In future, other filtration and dewatering technologies might be useful. The product, together with the liquid drained from the brewer's yeast canisters could be directly used as fertilizer on farmland. Yeast as a fertilizer was evaluated on corn (*Zea mays*), sugarcane (*Saccharum officinarum, S. spontaneum*), tomato (*Solanum lycopersicum*), and pine trees (*Pinus sylvestris*, *Armeniaca sibirica*), where results showed higher plant biomass production, higher nitrogen and P assimilation, and improved soil quality in comparison to plants without yeast fertilization [22, 51, 52]. Bio-stimulating effects of yeast as a fertilizer can be direct, such as the addition of soluble nutrients [53], production of different phytohormones and enzymes that support plant growth, and indirect mechanisms such as the mitigation of stress caused by high salinity, metal toxicity, or pH [54, 55]. Therefore, we believe that excess brewer's yeast [21] can be good microorganisms for P recovery to produce an environmentally friendly fertilizer as an end product that is safe for humans.

4.5 | Summary

At a pilot scale of 1 m^3, we demonstrated a three-stage P recovery process combining the production of a free P-rich water-phase in 3 h (stage 1), the production of P-depleted yeast in 48 h (stage 2a), the recovery of free P using P-depleted yeast in 3 h (stage 2b), and the production of P-rich yeast sludge in 1 h as fertilizer (stage 3). Stage 2a is independent of the other stages and can be carried out in a stand-alone procedure. Therefore, the core process takes 7 h. The development of green and environmentally friendly nutrient recovery processes will play an increasingly important role in the future. The pilot plant presented here is a step in this direction.

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CONFLICT OF INTEREST STATEMENT

The authors have declared no conflict of interest.

DATA AVAILABILITY STATEMENT

All data was made available upon manuscript submission in pdf format.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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