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# Production of bacterial nanocellulose (BNC) and its application as a solid support in transition metal catalysed cross-coupling reactions

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**Abstract**

Bacterial nanocellulose (BNC) emerged as an attractive advanced biomaterial that provides desirable properties such as high strength, lightweight, tailorable surface chemistry, hydrophilicity, and biodegradability.

BNC was successfully obtained from a wide range of carbon sources including sugars derived from grass biomass using *Komagataeibacter medellinensis* ID13488 strain with yields up to 6 g $L^{-1}$ in static fermentation. Produced BNC was utilized in straightforward catalyst preparation as a solid support for two different transition metals, palladium and copper with metal loading of 20 and 3 weight %, respectively. Sustainable catalysts were applied in the synthesis of valuable fine chemicals, such as biphenyl-4-amine and 4'-fluorobiphenyl-4-amine, used in drug discovery, perfumes and dye industries with excellent product yields of up to 99%. Pd/BNC catalyst was reused 4 times and applied in two consecutive reactions, Suzuki-Miyaura cross-coupling reaction followed by hydrogenation of nitro to amino group while Cu/BNC catalyst was examined in Chan-Lam coupling reaction. Overall, the environmentally benign process of obtaining nanocellulose from biomass, followed by its utilisation as a solid support in metal-catalysed reactions and its recovery has been described. These findings reveal that BNC is a good support material, and it can be used as a support for different catalytic systems.

**Keywords:** Bacterial nanocellulose; *Komagataeibacter medellinensis*; Cross-coupling reactions, Catalysis

## 1. Introduction

Cellulose is the most abundant bio-macromolecule on Earth and it is usually obtained from plant resources such as forest trees and cotton [1, 2]. It's superior properties including strength, lightweight, tailorable surface chemistry, renewability, biodegradability and no-toxicity are reason that this material has been produced at $10^{11}$ tonnes per year and that much effort is applied into advanced applications such as green electronics, biological devices, energy storage [3]. Striking properties have been observed when cellulose size has been decreased to the nanoscale. Nanoscale cellulose fibrils, with diameters from 5-60 nm are produced through mechanical grinding procedures, chemical treatments such as 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) catalysed oxidation or through combination of chemical and mechanical treatments with full life cycle assessment for large scale industrial production which was recently carried out [4, 5].

On the other side, certain microorganisms including species of *Gluconacetobacter*, *Komagataeibacter, Enterobacter, Rhodococcus* and *Sarcina* bacterial genera are capable of producing nanoscale cellulose through fermentative processes [6, 7]. Bacterial nanocellulose (BNC) exhibits unique properties and unlike nanocellulose obtained from plant sources, does not require pre-treatment to remove lignin and hemicellulose, as it is synthesized as pure cellulose with ultrafine network architecture, and characteristic ribbon-like microfibrils of 20-100 nm in diameter. Improved mechanical properties of BNC in comparison to plant derived one such as higher water holding capacity and hydrophilicity, have allowed even wider application of nanocellulose especially in the biotechnological, medical, pharmaceutical, and food industries [8]. More affordable production has advanced this material in the green chemistry sector [9, 10].

Catalysis plays a very important role in the synthesis of various industrially and biochemically important compounds especially having in mind environmental aspects [11,

12]. From a sustainable technology point of view, heterogeneous catalysts have several advantages in comparison to homogeneous catalysts such as simple handling and storage, recycling, cost effectiveness and ease of catalyst and product separation [13-15]. In recent years, application of nanocellulose as a transition metal catalyst support in cross-coupling reactions has attracted considerable attention [9, 16]. Bacterial nanocellulose was found to be able to catalyse the deposition of palladium within its structure to generate a fine and homogeneous catalyst layer for the first time in 2003 [17]. Ever since then, a lot of research effort has been devoted to the development and application of these sustainable heterogeneous catalysts, however they have not been applied commercially [18, 19].

*Komagataeibacter* genus is considered the most efficient cellulose producers, and the *K. xylinus* strain is considered to be representative model organism used for investigating BNC production [6, 20]. The effects of carbon sources and culture medium on the production of BNC were studied [21, 22]. Scale-up of the BNC production in submerged fermentation has recently been achieved using four different *Komagataeibacter xylinus* strains [23]. Moreover, waste materials such as agroindustrial residues were assessed for the efficient sustainable microbial production of nanocellulose [24, 25]. Despite efforts, commercial production of BNC has remained a challenge, primarily due to the strain properties of cellulose-producing bacteria and the specific fermentation conditions.

Herein, we have optimized the production of BNC utilising the *K. medellinensis* ID13488 strain using wide variety of carbon sources, and for the first time fermentable sugar mixture obtained by grass biomass hydrolysis opened the new possibility for biotechnologically obtaining nanocellulose from the plant biomass. In addition, produced BNC membranes were utilized as a solid support for two different transition-metal catalysed cross-coupling reactions. Pd/BNC catalyst was applied in two consecutive reactions, Suzuki-Miyaura cross-coupling reaction followed by hydrogenation of nitro to amino group while

Cu/BNC catalyst was examined in Chan-Lam coupling reaction. In this study we demonstrated the overall process starting from the production of BNC, characterization of the biopolymeric material, and its utilisation as a solid support for transition-metal catalysts, along with the life-cycle of the catalytic systems.

## 2. Materials and Methods

### *2.1. General instrumentation and chemicals*

Dry-flash chromatography was performed on $SiO_2$ (0.018–0.032 mm). Melting points were determined on a Boetius PMHK apparatus and were not corrected. IR spectra were recorded on a Thermo-Scientific Nicolet 6700 FT-IR Diamond Crystal instrument. $^{1}H$ and $^{13}C$ NMR spectra were recorded on a Bruker Ultrashield Avance III spectrometer (at 500 and 125 MHz, respectively) using $CDCl_3$ as the solvent and tetramethylsilane (TMS) as an internal standard. Chemical shifts are expressed in parts per million (ppm) on the (δ) scale. Chemical shifts were calibrated relative to those of the solvent. GC–MS spectra of the synthesized compounds were acquired on an Agilent Technologies 7890A apparatus equipped with a DB-5 MS column (30 m × 0.25 mm × 0.25 μm), a 5975C MSD and FID detector. The selected values were as follows: carrier gas was He (1.0 mL/min), temperature linearly increased from 40–315 °C (10 °C/min), injection volume = 1 μL, temperature = 250 °C, temperature (FID detector) = 300 °C, and EI mass spectra range: m/z 40–550. IR, $^{1}H$ NMR, $^{13}C$ NMR and GC–MS analyses of synthesized compounds are provided in Supplementary data.

All media components including peptone, yeast extract, agar, sugars, citric acid, and inorganic salts were purchased either from Oxoid (Cambridge, UK), Becton Dickinson (Sparks, MD, USA), or Sigma Aldrich (Munich, Germany). All chemicals and solvents were

supplied by Sigma Aldrich (Munich, Germany). Ultra-pure water was prepared by Millipore Simplicity 185 System.

### *2.2. Bacterial nanocellulose (BNC) production by Komagataeibacter medellinensis*

Nanocellulose producing strain *Komagataeibacter medellinensis* ID13488 has been previously described [20, 26] and was kindly provided by Professor Orlando Rojas from the School of Chemical Engineering, Aalto University, Finland (this strain has also been deposited as: NBRC 3288 (Nite Biological Research Center culture collection), LMG 1693 (Belgian Coordinated Collections of Microorganisms Bacteria) and IFO 3288 (Institute for fermentation Osaka). The strain was cultivated in a standard Hestrin–Schramm medium (HS) containing 20 g $L^{-1}$ glucose, 5 g $L^{-1}$ peptone, 5 g $L^{-1}$ yeast extract, 2.5 g $L^{-1}$ $Na_2HPO_4$ and 1.15 g $L^{-1}$ citric acid [27], at 28 °C for four days under static conditions, and used as inoculum (1.5%, v/v) for further experiments. BNC was generally produced under static conditions for 8 or 10 days at 28 °C in HS medium. Erlenmeyer flasks (250 mL volume) containing 50 mL production media (approximately 50 $cm^2$ air-liquid surface) were used for BNC production optimization experiments. BNC was rinsed with water and treated for 16 h with 5% KOH aqueous solution, washed with deionized water until pH of 7.0 was reached, oven-dried at 65 °C until constant mass and weighted. For BNC production optimization experiments, the effect of duration of incubation (2, 4, 6, 8 and 10 days), different pH (2.5, 3.5, 4.5, 5.5 and 7.5; buffered with citric acid buffer) and different sugar concentration (1%, 2%, 3% and 4%, w/v) on BNC yield was assessed.

In order to assess the influence of different carbon sources on the production of BNC glucose in HS medium (pH 4.5, 8 days at 28 °C) was replaced with either of the following: maltose, lactose, mannitol, sorbitol, starch, mannose, arabinose, xylose or ethanol. Mixture of glucose, xylose, arabinose and mannose (77%, 18.5%, 2.5% and 2%, respectively) was also used to test *K. medellinensis* ability to grow and produce nanocellulose on mixture of

fermentable sugars obtained by hydrolysis of grass biomass [28]. Carbon-source concentration was calculated to the same amount (8 g $L^{-1}$) of carbon as in 20 g $L^{-1}$ glucose. Sugars were filter-sterilized as 50% (w/v) and added after sterilization. Growth and BNC production experiments were done in duplicate on two separate occasions.

### *2.3. Preparation BNC supported catalysts*

Bacterial nanocellulose discs, obtained from the *K. medellinensis* growth on 2% glucose in 30 mL bottles of 1.8 mm diameter (approximately 2.5 $cm^2$ air-liquid surface area) were treated with KOH, extensively washed with deionized $H_2O$ until pH of 7.0 was reached and were dried under vacuum at 50 °C for 3 h.

#### *2.3.1. Preparation of Pd/nanocellulose (Pd/BNC)*

For the preparation of Pd/BNC previously reported procedure was followed.[29] Briefly, to a suspension of BNC (44 mg) in $H_2O$ (3 mL) $PdCl_2$ (14.4 mg, 0.082 mmol) was added and the reaction mixture was degassed. The reaction mixture was heated at 140 °C for 0.5 h under argon atmosphere. The suspension was cooled to room temperature (25 °C) and a solution of $NaBH_4$ (106 mg, 2.8 mmol) in $H_2O$ (2 mL) was added to the reaction mixture. The resulting mixture was stirred (1100 rpm) and heated at 140 °C. After 5 h reaction mixture was centrifuged and washed several times with deionized $H_2O$. The resulting material was dried under vacuum at 50 °C to obtain Pd/BNC (47 mg).

#### *2.3.2. Preparation of Cu/nanocellulose (Cu/BNC)*

For the preparation of Cu/BNC previously reported procedure was followed.[30] Briefly, to a suspension of BNC (23 mg) in $H_2O$ (1 mL) $Cu(NO_3)_2 \cdot 3H_2O$ (8.7 mg, 0.036 mmol) was added and the reaction mixture was degassed. The reaction mixture was heated at 70 °C under argon atmosphere. The suspension was cooled to room temperature (r.t.) and $N_2H_4 \cdot H_2O$ (19.2 µL, 0.40 mmol) was added to the reaction mixture. The resulting mixture

was stirred (1100 rpm) and heated at 70 °C. After 24 h reaction mixture was centrifuged and washed several times with deionized $H_2O$. The resulting material was dried under vacuum at 50 °C to give Cu/BNC (21 mg).

### *2.4. Characterization of Pd/BNC and Cu/BNC catalysts*

Concentration of Pd and Cu within Pd/BNC and Cu/BNC was determined using inductively coupled plasma-quadrupole mass spectrometry (ICP-QMS; iCAP Q, Thermo Scientific X series 2). Entire system of instrument was controlled with Qtegra Software. Samples were dissolved in 1 mL 14 M $HNO_3$ and transferred to volumetric flask. The final volume was 50 mL. Single-element stock solution of Cu (1.0 g $L^{-1}$) and Pd (10.0 g $L^{-1}$) were used for preparation of intermediate standard solutions. Instrument operating conditions for determination of Cu and Pd: RF power (W) = 1548; Gas flows (L $min^{-1}$) = 13.9, 1.09, 0.8; Acquisition time = 3 × 50 s; Points per peak = 3; Dwell time = 10 ns; Detector mode was analog/pulse; Replicates = 3. Measured isotope (normal mode) = $^{63}Cu$, $^{65}Cu$, $^{104}Pd$, $^{105}Pd$, $^{106}Pd$, $^{108}Pd$, $^{110}Pd$. The same instrument and protocol were used for the determination of Pd leaching after the completion of reactions.

The morphology of the obtained BNC, Pd/BNC and Cu/BNC samples were examined using a high resolution field emission scanning electron microscope (Carl Zesis ULTRA plus, Oberkochen, Germany) using InLens detector with an accelerating voltage of 5 kV at working distance of 5 mm. Prior to characterization, the vacuum dried samples were coated with gold/palladium (80/20 ratio) for 10 sec using a Cressington 208HR high resolution sputter coater. Elemental composition of the samples surface was identified using energy-dispersive X-ray spectroscopy (EDX) analysis (Oxford Instruments, Abingdon, United Kingdom).

Thermogravimetric analysis (TGA) of samples were carried out using a Perkin Elmer PYRIS$^{TM}$ 1 thermal analyzer. The samples were heated from 30 $^{o}$C to 800 $^{o}$C with a scanning rate of 10 $^{o}$C min$^{-1}$ under an inert ($N_2$) atmosphere.

The structural analysis of the samples was evaluated by X-ray diffraction (XRD) using a Bruker D8 advanced diffractometer with a step of 0.082$^{o}$ with Cu Kα radiation source (λ=1.54060 Å) operating at 40 kV and 40 mA. The XRD patterns were obtained over the angular range 2θ from 10 to 70$^{o}$.

### *2.5. Application of Pd/BNC and Cu/BNC catalysts in organic synthesis*

#### *2.5.1. General procedure for Suzuki-Miyaura coupling reaction using Pd/BNC*

*Biphenyl (**3a**)* [31]: Pd/BNC has been applied in synthesis of biphenyl (**3b**) using ligand-free methodology. To a dry glass reaction tube purged with argon bromobenzene **1a** (78 μL, 0.743 mmol), phenylboronic acid **2a** (134 mg, 1.12 mmol), $K_2CO_3$ (308 mg, 2.23 mmol), TBAB (240 mg, 0.743 mmol), Pd/BNC (1 mg (0.2 mg [Pd]), 0.25 mol %) and $H_2O$ (5 mL) were added. The reaction mixture was purged with argon and heated at 90 °C for 16 h. The solution was cooled to r.t. and filtered. The Pd/BNC was washed with ethyl acetate and then deionized water. The organic solvent was removed under reduced pressure and the crude product was purified by dry-flash chromatography ($SiO_2$: hexane/EtOAc = 95/5) to yield **3a** 130 mg (87%) as a colorless solid.

*4-Methoxybiphenyl* (***3b***): Pd/BNC was also utilized to obtain 4-methoxybiphenyl (**3b**) in water, using ligand-free methodology [31]. Following the general procedure for Suzuki reaction, compound **3b** (106 mg, 77%) was obtained as a colorless solid.

*4-Nitrobiphenyl (**3c**):* Pd/BNC was also utilized to obtain 4-nitrobiphenyl (**3c**) using ligand-free methodology [32]. Following the general procedure for Suzuki reaction, compound **3c** (128 mg, 86%) was obtained as a yellow solid.

*4-Fluoro-4'-nitrobiphenyl (**3d**)*: Pd/BNC was also utilized to obtain 4-fluoro-4'-nitrobiphenyl (**5**) in water, using ligand-free methodology [33]. Following the general procedure for Suzuki reaction, compound **3d** (185 mg, 91%) was obtained as a yellow solid.

*2.5.2. Reusability test of catalyst for Pd/BNC catalysed Suzuki-Miyaura reaction between 1-bromo-4-nitrobenzene 1b and phenylboronic acid 2a*

After completion of the reaction between 1-bromo-4-nitrobenzene and phenylboronic acid, the catalyst was removed from the reaction mixture by a forceps and was washed with water and ethyl acetate, and dried under vacuum with heating at 50 °C until a complete dry mass was formed. The formed activated residue containing Pd/BNC catalyst was used for the second and further reaction cycles following the general reaction procedure.

*2.5.3. Mercury poisoning experiments*

Mercury poisoning test was conducted according to previous study [34] whereby the Pd/BNC catalysed Suzuki-Miyaura reaction between 1-bromo-4-nitrobenzene **1b** and phenylboronic acid **2a** was performed in the presence of a 300 molar equivalents of mercury.

*2.5.4. General procedure for the reduction of nitro group using Pd/BNC*

Pd/BNC was subsequently used in procedure for reduction of nitro group to obtain biphenyl-4-amine (**4**) and 4'-fluorobiphenyl-4-amine (**5**).[35]

*Biphenyl-4-amine (**4**)*: To a solution of compound **3c** (45 mg, 0.23 mmol) in ethanol (1.8 mL) and hexane (0.2 mL) Pd/BNC (5.3 mg (1.1 mg [Pd]), 10 mol %) was added and the reaction mixture was purged with hydrogen. The resulting mixture was stirred at r.t. in hydrogen atmosphere for 3 h. The reaction mixture was filtered and the solvent was removed under reduced pressure to yield **4** 32 mg (99 %) as a white solid.

*4'-Fluorobiphenyl-4-amine (**5**)*: Following the general procedure for reduction, compound **5** (55.6 mg, 99 %) was obtained as a white solid.

*Procedure for Chan–Lam coupling reaction using Cu/BNC catalyst*

*N*-benzylaniline (**7**) was obtained using amine (**6**) and Cu/BNC catalyst at room temperature [36]. Briefly, to a solution of amine **6** (17 mg, 0.16 mmol) in methanol (0.5 mL) Cu/BNC (5 mg (0.15 mg [Cu]), 1.5 mol %) and **2a** (13.5 mg, 0.11 mmol) were added. The resulting mixture was stirred at r.t. for 16 h. The reaction mixture was filtered and solvent was removed under reduced pressure and the crude product was purified by dry-flash chromatography ($SiO_2$: $CH_2Cl_2$) to yield **7** 9.5 mg (48 %) as an oil.

### *2.6. Statistical analysis*

Statistical significance was determined by unpaired two-tailed Student's *t*-test ($p \leq 0.05$).

## 3. Results and discussion

### *3.1. Optimisation of BNC production*

*Komagataeibacter medellinensis* ID13488 strain has been previously isolated and described in 2012 as an acid tolerant *Gluconacetobacter medellinensis* ID13488 from the fermented vinegar from local markets in Medellin, Colombia as Gram-negative rods, approximately 1–3 µm long and and 0.6 – 0.7 µm wide, capable of producing 2- and 5- keto-D-gluconic acid from D-glucose [26]. The strain has been subsequently transferred and renamed to *Komagataeibacter* genus [20]. D-Glucose, ethanol, D-fructose, maltose, sucrose, sorbitol, D-mannitol, D-ribose and D-xylose were reported as suitable carbon sources for this strain [26]. The cellulose producing ability of this strain has been assessed on different occasions [22, 37, 38]. However, reports on BNC production and the optimal conditions were not consistent, possibly due to the differing fermentation conditions, thus we have carried out the optimization study, using reported parameters as a starting point. Firstly, time course experiment was carried out in the static fermentation utilizing Hestrin–Schramm (HS)

medium (pH 4.5, 28°C) and 2% glucose as carbon source. It was determined that BNC production was considerable after two days of static incubation (1.8 g $L^{-1}$) and it doubled within further two days. The average increase of BNC amount was 0.38 g $L^{-1}$ per day from the 4$^{th}$ to the 10$^{th}$ day of incubation (Fig. 1a). Further incubation resulted in the decrease of BNC yield as by 12$^{th}$ day of incubation almost plateau was reached (data not shown). This was in slight discrepancy with previous report when the plateau has been reached after 8$^{th}$ day of incubation [37], but in accordance with the more recent report when the plateau has been reached after 10 days of static incubation [22] for this strain. Overall, in our study 20 g $L^{-1}$ glucose was converted to 6 g $L^{-1}$ BNC which was 30% conversion and between 1.2- to 1.5-fold higher product to substrate yield in comparison to previous reports using 2% glucose as substrate with this strain for the same fermentation period [22, 37]. Improved yields may be due to different inoculum used, which was determined as an important factor in overall BNC yields [39]. In our experiments, 1.5% (v/v) inoculum of the 4-day old culture from HS medium containing 2% glucose as a carbon source has been used. Findings from our study are in good agreement with recently described strain *K. rhaeticus* P 1463 isolated from Kombucha that was converting sugars to BNC at 37% [39].

The effect of pH of the production medium on BNC yields was also evaluated confirming that *K. medellinensis* ID13488 was indeed able to grow and produce BNC in a wide-range of pH values, with the yields of BNC being comparable between pH 3.5-5.5 (Fig. 1b). At pH values of 2.5 and 7.5 the BNC yields were on average 1.3- and 3.2-folds lower, respectively in comparison to average yields obtained at pH 3.5-5.5. Castro et al. found the pH optimum at 3.5 for this strain [37]. The initial concentration of the glucose in the production medium was determined to be the important factor for BNC production (Fig. 1c), with product to substrate ratios decreasing from 0.39 to 0.16 using 1% and 4% glucose as carbon source. Higher decrease of BNC production of almost 2-fold has been reported for

this strain at 3% of glucose [22], while similar decrease of BNC production at 4% glucose was previously reported for *K. rhaeticus* P 1463 [39].

The optimal conversion rate coupled with absolute BNC yields in this study was established for 2% glucose, pH 4.5 and incubation of 10 days. These optimal conditions were utilised to assess the broad spectrum of carbon sources for BNC production (Fig. 2a). For the first time, mixture of sugars described as a product of grass biomass treatment [28] was successfully used as a substrate for BNC production, with yields comparable to that obtained with pure glucose as a carbon source (Fig. 2a). Given that about 69% of total agricultural land area are grasslands, which are low in greenhouse gas emissions, it is estimated that they can contribute significantly for biorefinery feedstocks without compromising food and feed sectors [40]. Conversion of grass biomass derived sugars to valuable biopolymer, such as BNC, represents additional avenue for valorisation of non-food renewable biomass based starting materials. This substrate has been previously successfully converted to a different type of bacterial biopolymer, polyhydroxyalkanoates [28]. Importantly, BNC was produced from additional 9 carbon sources, with mannose and starch also found to be comparable to glucose, and the lowest yields detected for ethanol as a substrate (Fig. 2a). Indeed, it has been shown recently that the addition of ethanol to the HS medium already containing glucose, positively affected overall BNC yield but negatively affected the crystallinity, degree of polymerization, and degradation temperature in fermentation of *K. medellinensis* ID13488 [38]. In comparison, BNC yields obtained with other *Komagataeibacter* strains included 3.34 g $L^{-1}$ using the medium containing mannitol and corn steep liquor [41] while 5.6 g $L^{-1}$ and 8.2 g $L^{-1}$ of BNC were produced when *K. hansenii* UAC09 was cultivated in a medium with coffee cherry husk and corn steep liquor as substrates [42]. When *K. rhaeticus* strain P 1463 was cultivated in the medium containing apple juice with a carbon substrate concentration of 20 g $L^{-1}$, 4.77 g $L^{-1}$ of BNC was obtained [39].

### *3.2. Application of BNC supported metal catalysts*

The use of nanocellulose as a transition metal catalyst support is appealing due to its high stability, high surface area and insolubility in common solvents, particularly water. Therefore, BNC produced by *K. medellinensis* ID13488 was evaluated as a support for Pd and Cu catalysts. The Pd/BNC catalyst was prepared by mixing of palladium-chloride, BNC and sodium borohydride in water at 140 °C (Scheme 1), following the procedure described in the literature [29]. Similarly, Cu/BNC catalyst was obtained by mixing of copper(II) nitrate trihydrate, hydrazine hydrate as a reducing agent and BNC in water at 70 °C (Scheme 1). The amount of the palladium supported on Pd/BNC was 20.55 wt% and the amount of the copper supported on Cu/BNC was about 10-fold lower in comparison to that of palladium, being 2.98 wt%, as determined by ICP-QMS.

The morphology of BNC, Pd/BNC and Cu/BNC samples were observed using scanning electron microscopy (Fig. 3). The surfaces of all BNC samples were relatively smooth, while deposits of metal catalysts were visible on the surface of Pd/BNC and Cu/BNC samples adding to the roughness of the surface (Fig. 3b and 3c). At higher magnification, 3D-network structures with randomly oriented nano-fibrils were observed in all BNC samples (Fig. 3 d-i). However, difference in metal catalyst's distribution between Pd/BNC and Cu/BNC were observed. Cu catalyst appeared to be in spherical structure and evenly distributed all over the surface (Fig. 3f and 3i), while Pd where incorporated in to the BNC matrix (Fig. 3e and 3h).

ICP-QMS analysis revealed a 10-fold increase in amount of Pd support on Pd/BNC compared to Cu support. As a result, higher amount of Pd catalyst were dispersed throughout Pd/BNC matrix while in the case of Cu/BNC, metal catalyst predominantly appeared only on the surface. It can be indicated that the process of Pd catalyst incorporation in to BNC matrix is comparatively more efficient than Cu catalyst. It is evident from the Fig. 3g-i that nano-

fibrils were in the range of 30 nm to 130 nm in width and Cu catalyst found on the BNC surface had the particle size in the range of 140 nm to 250 nm with an average particle size of 177 nm.

EDX analysis coupled with scanning electron microscope was performed to detect elemental composition of confirmed metal/BNC samples (Fig. 4). As expected, the EDX spectrums of Pd/BNC (Fig. 4a) and Cu/BNC (Fig. 4b) reveals the existence of Pd and Cu, which confirms the presence of metal catalysts on the BNC matrix. Although SEM analysis indicated that Pd catalyst was predominantly incorporated within nanocellulose matrix, SEM-EDX analysis exposed that the agglomerated Pd catalyst were also found on the certain areas of Pd/BNC surface (Fig. 4a). Thermogravimetric analysis (TGA) and its derivative curves of Cu/BNC and Pd/BNC are provided in Fig. 4c and 4d. The initial weight loss of ~5% was due to the evaporation of loosely bound moisture on the surfaces of the materials. From TGA analysis it was evident that BNC modified with metal catalyst showed the higher amount of residue, indicating the presence of metal catalysts in M/BNC samples. Both metal catalysts had a single major weight loss between 300 ºC - 400 ºC and the onset temperature of Pd/BNC was lower than Cu/BNC, while the peak temperatures from derivative graphs indicated that Cu/BNC had higher peak temperature than Pd/BNC (Fig. 4d).

In order to explore the scope of the Pd/BNC catalysed Suzuki-Miyaura reaction, a variety of aryl halides and aryl boronic acids were investigated. The results are summarized in Table 1. As expected, the coupling reaction between phenylboronic acid and bromobenzene gave higher yield over the reaction of phenylboronic acid with iodobenzene and chlorobenzene, respectively. In addition, the reactions of bromobenzene with 4-methoxyphenylboronic acid led to the formation of desired product in good yield. Nitro- and amino-substituted biphenyls have been extensively used as precursors in drug discovery, perfumes and dye industries [43, 44]. Therefore, prepared Pd/BNC catalyst has been also

applied in Suzuki-Miyaura cross-coupling reaction in water for the preparation of nitro compounds **3c** and **3d** in high to excellent yields of 86% and 91%, respectively (Table 1).

In order to get insight into the nature of the catalyst a mercury poisoning test of the reaction between 1-bromo-4-nitrobenzene and phenylboronic acid was performed [34]. In the presence of a 300 molar equivalents of mercury, relative to the Pd catalyst, the yield of 4-nitrobiphenyl **3c** decreased from 86% to 23% which clearly indicated that the presented transformation was heterogeneously catalysed. Reusability is very important property of the heterogeneous catalyst from the practical, synthetic and environmental point of view. After completion of the reaction between 1-bromo-4-nitrobenzene and phenylboronic acid, the catalyst was removed from the reaction mixture by a forceps and was washed with water and ethyl acetate, and dried under vacuum with heating at 50 °C until a complete dry mass was formed before being used for the next cycle of reaction (Fig. 5). It was found that the Pd/BNC catalyst could be reused four times without a significant change in activity (Fig. 5a). Metal leaching was studied by ICP-QMS analysis after the first reaction cycle. It was found that the concentration of palladium in the filtrate remaining after the separation of the catalyst was 0.013 ppm, indicating that there was no significant amount of leaching. After the 4 reaction cycles, Pd/BNC was examined by SEM (Fig. 5b and 5c). SEM analysis confirmed the presence of Pd metal catalyst on the BNC matrix in BNC fibres even after the recycling of the catalyst. However, XRD analysis showed that after 4 consecutive reaction cycles, Pd/BNC catalyst is becoming amorphous and its thermogravimetric properties also slowly declined (Supporting Material, Fig. S3 and Fig. S4).

TONs and TOFs were calculated for all Suzuki-Miyaura cross-coupling reaction performed in this study (Table 1). For the purpose of comparison TONs and TOFs of Suzuki-Miyaura cross-coupling reaction catalysed by various Pd-supported catalysts reported in literature are summarised in Table 2. Generally, agar- and starch supported Pd catalysts under

solvent free conditions yielded highest TONs and TOFs, while in polar media (i. e. $H_2O$ and EtOH) TONs and TOFs were usually lower. While TONs and TOFs of BNC supported Pd catalyst generated in this study were considerably lower in comparison to solid-supported Pd catalysts used under solvent free conditions, they were comparable or even better than TONs and TOFs obtained in reactions when Pd/$TiO_2$ nanoparticles, Au/Pd bimetallic nanoparticles, biopolymer–metal complex wool-Pd or Pd/C were used in polar solvent (Table 3).

The application of Pd/BNC catalyst was further extended to reduction of nitro to amino group with hydrogen (Scheme 2). Notably, quantitative yields of amines **4** and **5** were obtained without any additional purification methods. The application of the sustainable catalyst allowed its easy recovery and utilisation in two consecutive reactions of a different type.

The application of Cu/BNC catalyst was examined in Chan-Lam coupling reaction for the synthesis of secondary amine **7** (Scheme 3), a valuable synthetic intermediate for the preparation of a number important biologically active derivatives [45, 46]. Even though the yield of compound **7** was moderate, which could be due to the lower incorporation of Cu and its predominant distribution on the surface of the BNC, this opened the room for the further optimisation study. Previously, copper nanoparticles were efficiently loaded on the surface of pure cellulose acetate and showed remarkable catalytic properties for the reduction of 4-nitro phenol [16] while copper nanoparticles were also successfully generated inside cellulose matrix using *Terminalia catappa* leaf extract [47].

Importantly, the choice of solvents for the organic synthesis allowed easy separation of both BNC catalysts prepared in this study by filtration and their subsequent reuse.

## 4. Conclusions

BNC was biotechnologically produced in static fermentations of acid-tolerant *K. medellinensis* strain. Optimal conditions and high BNC yields were achieved using variety of carbon sources including sugars derived from grass biomass hydrolysis. Produced BNC was shown to be excellent solid support for palladium and copper. Pd/BNC catalyst was applied in two consecutive reactions, Suzuki-Miyaura cross-coupling reaction followed by hydrogenation of nitro to amino group obtaining products in 86-99% yields. Cu/BNC catalyst was examined in Chan-Lam coupling reaction, obtaining *N*-benzylaniline a valuable synthetic intermediate for the preparation of a number of important biologically active derivatives. Utilisation of sustainable BNC-based catalysts afforded the ease of separation and the reuse of the catalysts. These findings represent valuable addition towards efforts for wider utilization of biomaterial and implementation of cleaner production processes.

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## Supplementary data

Supplementary data related to this article are provided.

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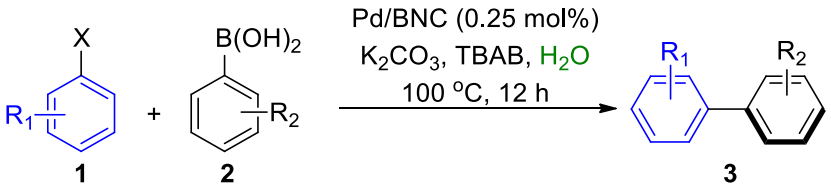
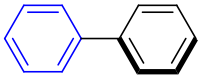
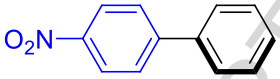
**Table 1.** Pd/BNC catalysed Suzuki-Miyaura reaction of aryl halogenides with arylboronic acid in water.[a]



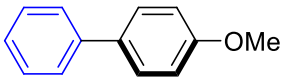
**3a**

| | | TON | TOF |
|---|---|---|---|
| I | **78%** | *312* | *26* |
| Br | **87%** | *352* | *29* |
| Cl | **14%** | *56* | *4.5* |



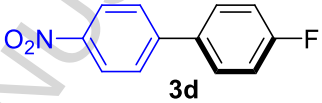

**3c**

| | | TON | TOF |
|---|---|---|---|
| Br | **86%** | *344* | *28.6* |
| Br | **23%**[b] | *92* | *7.6* |




**3b**

| | | TON | TOF |
|---|---|---|---|
| Br | **77%** | *308* | *26* |




**3d**

| | | TON | TOF |
|---|---|---|---|
| Br | **91%** | *372* | *31* |

[a]Reaction condition: **1** (0.74 mmol), **2** (1.12 mol), Pd/BNC (0.25 mol% (Pd)), $K_2CO_3$ (2.23 mmol), TBAB (0.74 mol), 5 mL $H_2O$, 100 °C, 16 h. [b]In the presence of excess Hg (Pd : Hg = 1: 300)

**Table 2.** Solvents, TONs and TOFs of the Pd catalysts on different solid supports for Suzuki-Miyaura C-C cross-coupling reactions.

| Pd/supported catalyst | Solvent | TON | TOF | Ref. |
|---|---|---|---|---|
| Chitosan-based Pd(II) catalyst | solvent free | 16400 | 205000 | [48] |
| Cellulose Schiff base supported Pd(II)catalyst | solvent free | 18000 | 180000 | [49] |
| Chitosan supported Pd catalyst | solvent free | 4200 | 36636 | [50] |
| Chitosan/cellulose-Pd(II) catalyst | solvent free | 5600 | 70000 | [51] |
| Agar-supported Pd catalyst | solvent free | 32666 | 326660 | [52] |
| Pd/$TiO_2$ nanoparticles | NMP/$H_2O$ | 137 | 34 | [53] |
| Starch-supported Pd catalyst | solvent free | 23000 | 287500 | [54] |
| Au/Pd bimetallic nanoparticles | EtOH/$H_2O$ | 22 | 0.9 | [55] |
| Pd/$Fe_3O_4$ nanoparticles | MeOH | 950 | 53 | [56] |
| Chitosan Schiff base supported Pd(II) catalysts | solvent free | 15167 | 189597 | [57] |
| Glyoxal and chitosan supported Pd(II)catalyst | toluene | 4600 | 383 | [58] |
| Nano-$Fe_3O_4$@$SiO_2$ supported Pd(0) | EtOH/$H_2O$ | 2900 | 2500 | [59] |
| Biopolymer–metal complex wool-Pd | $H_2O$ | 164 | 9 | [60] |
| Pd/C | EtOH/$H_2O$ | 29 | 14 | [32] |
| Pd supported on bacterial nanocellulose | $H_2O$ | 344 | 29 | This study |

**Figure legends**

**Fig. 1** Bacterial nanocellulose (BNC) production by *K. medellinensis* ID13488 utilising glucose as carbon source A) over 10 days static incubation in Hestrin–Schramm (HS) medium (pH 4.5, 28 °C); B) over 8 and 10 days at different pH values of HS medium; and C) over 8 and 10 days at pH 4.5 utilising different amount of glucose. (*Experiments were carried out in duplicate on two different occasions and differences were considered significant if $p \leq 0.05$.)

**Fig. 2** BNC production from variety of carbon sources utilised at 8 g $L^{-1}$ of carbon atoms by *Komagataeibacter medellinensis* ID13488 under static incubation for 10 days at 28 °C (A). Photographs of BNC discs formed at the air-liquid interphase utilizing two selected carbon sources (B). (*Experiments were carried out in duplicate on two different occasions and differences were considered significant if $p \leq 0.05$.)

**Fig. 3** Representative SEM micrographs of BNC (a, d, g), Pd/BNC (b, e, h) and Cu/BNC (c,f, i).

**Fig. 4** EDX analysis of Pd/BNC (a) and Cu/BNC (b) catalysts and TGA thermograms of both catalysts (c) and their derivatives (d) in temperature.

**Fig. 5** Recycling of Pd/BNC (a) and SEM analysis of the catalyst after 4 reaction cycles (b, c).

**Scheme 1.** Experimental approach to obtain and apply BNC as solid support in transition metal catalysed cross-coupling reactions.

**Scheme 2.** Reduction of nitro-group catalysed by Pd/BNC

**Scheme 3.** Chan-Lam coupling reaction catalysed by Cu/BNC

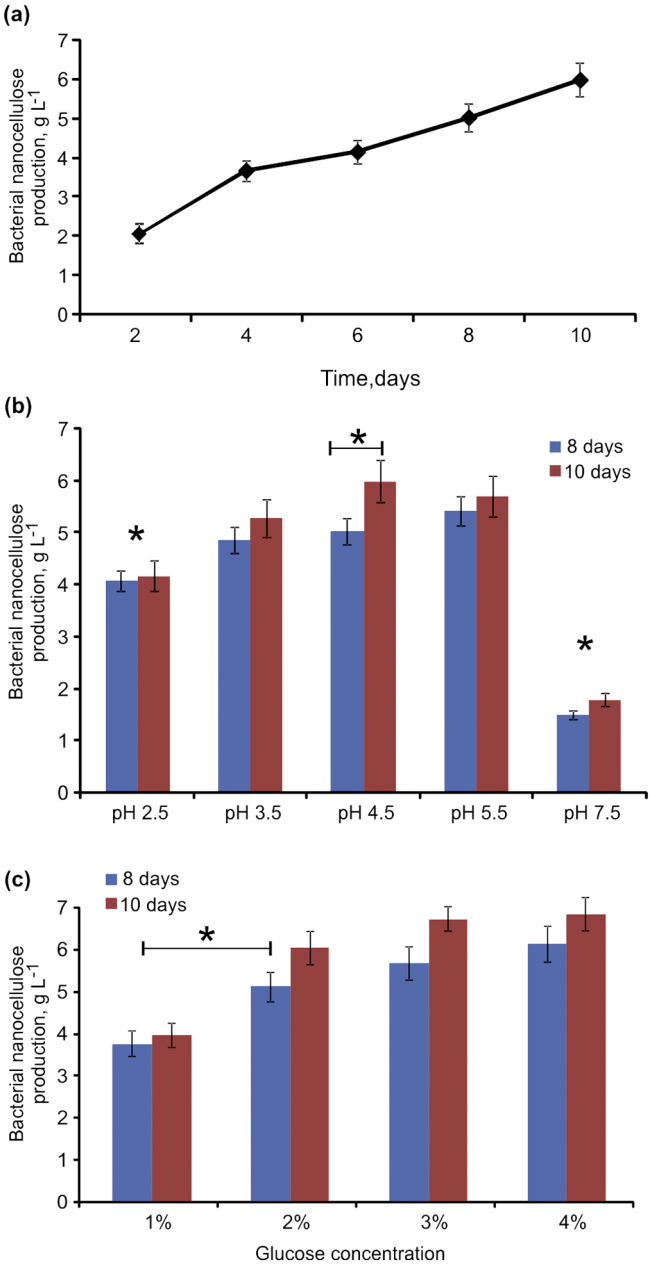

(a)
Bacterial nanocellulose production, g L-1
0
1
2
3
4
5
6
7
2
4
6
8
10
Time,days
(b)
Bacterial nanocellulose production, g L-1
8 days
10 days
*
*
*
pH 2.5
pH 3.5
pH 4.5
pH 5.5
pH 7.5
(c)
8 days
10 days
Bacterial nanocellulose production, g L-1
*
1%
2%
3%
4%
Glucose concentration

Figure 1

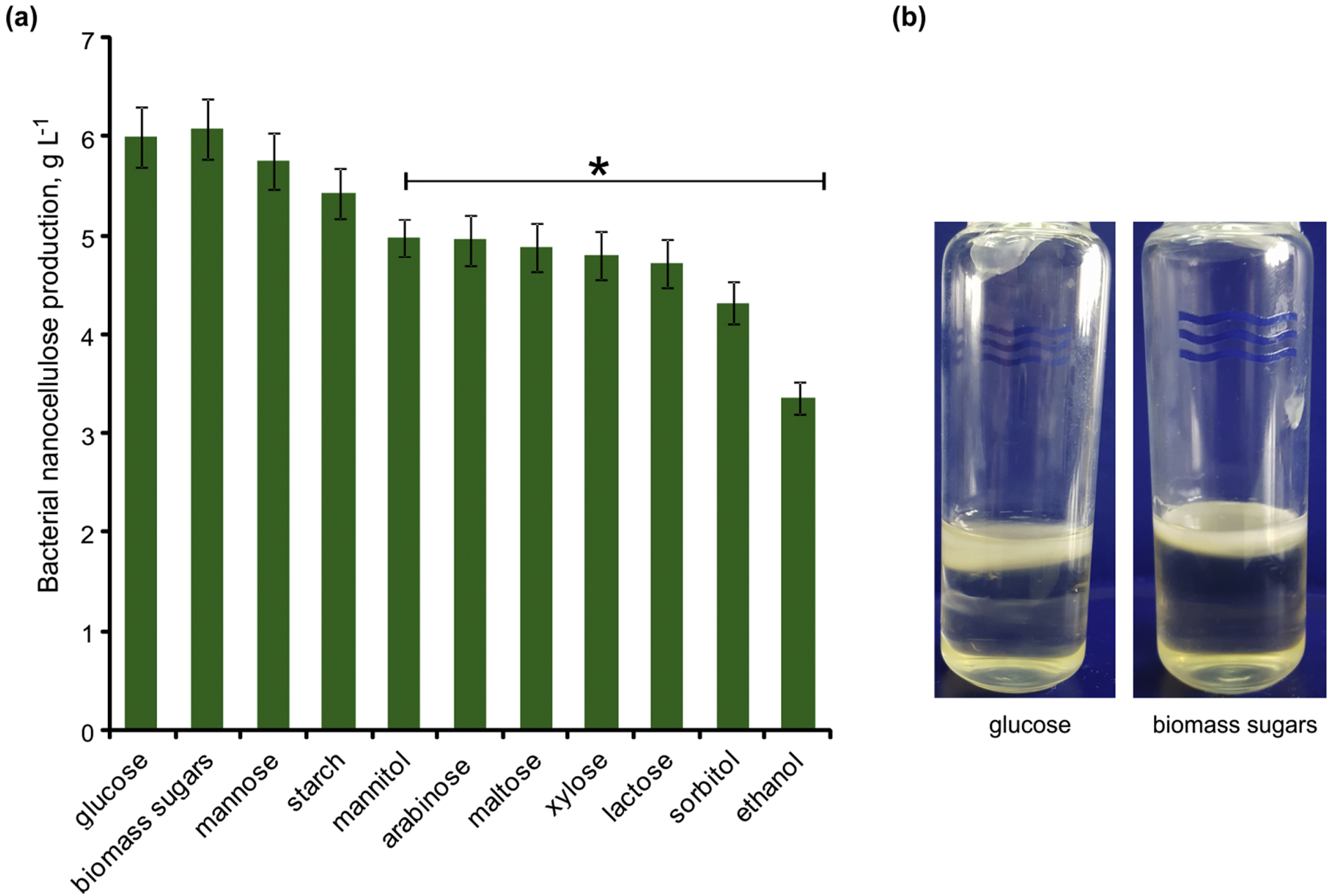

(a)
Bacterial nanocellulose production, g L-1
0
1
2
3
4
5
6
7
*
glucose
biomass sugars
mannose
starch
mannitol
arabinose
maltose
xylose
lactose
sorbitol
ethanol
(b)
glucose
biomass sugars

Figure 2

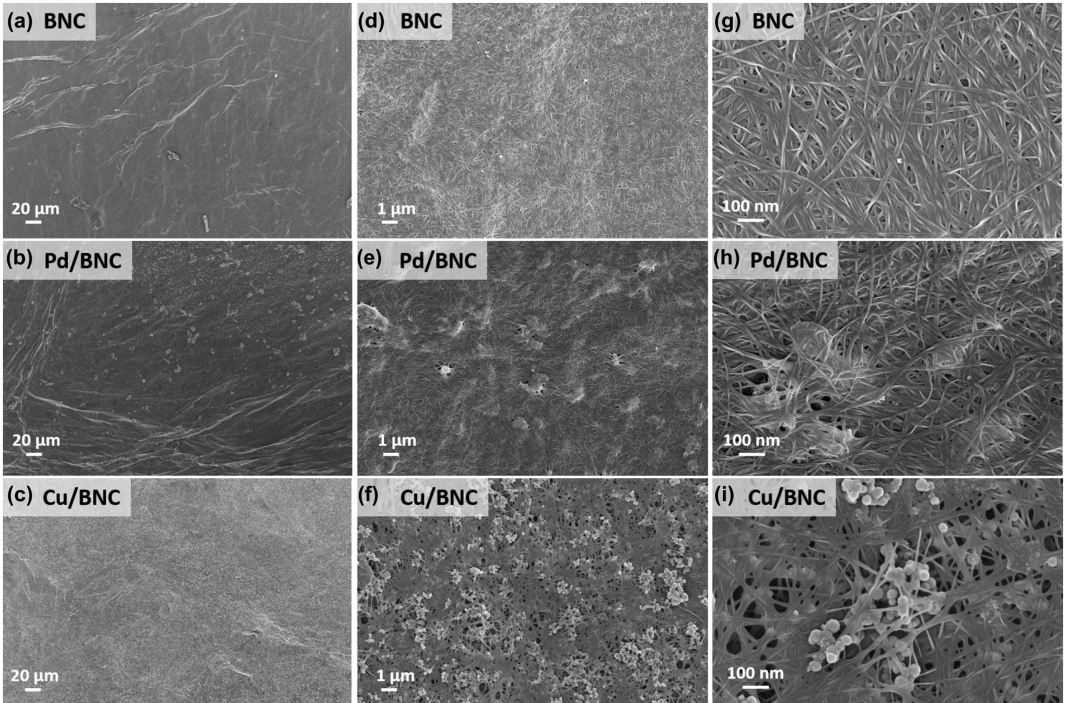

(a) BNC
20 μm
(d) BNC
1 μm
(g) BNC
100 nm
(b) Pd/BNC
20 μm
(e) Pd/BNC
1 μm
(h) Pd/BNC
100 nm
(c) Cu/BNC
20 μm
(f) Cu/BNC
1 μm
(i) Cu/BNC
100 nm

Figure 3

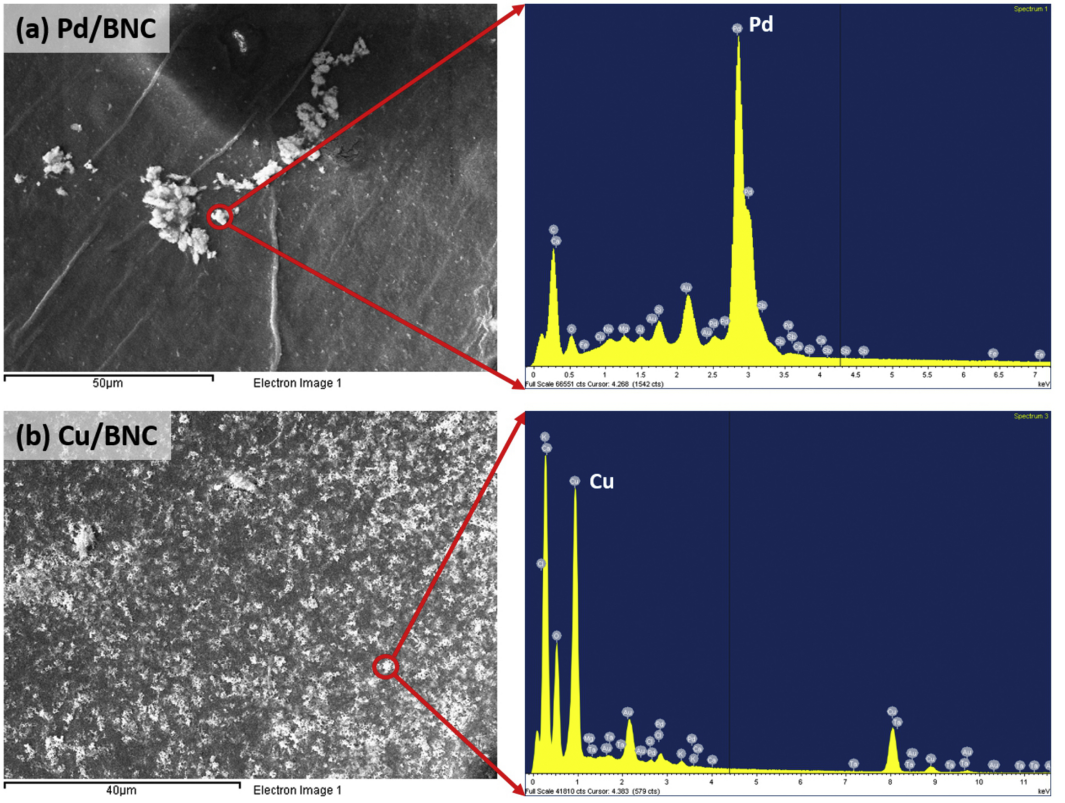

(a) Pd/BNC
50µm
Electron Image 1
Pd
(b) Cu/BNC
40µm
Electron Image 1
Cu
(c)
Pd/BNC
Cu/BNC
Onset temperatures:
Pd/BNC – 313 °C
Cu/BNC – 329 °C
Weight % (%)
Temperature (°C)
(d)
Peak temperatures:
Pd/BNC – 338 °C
Cu/BNC – 357 °C
Derivative Weight % (%/min)
Temperature (°C)

Figure 4

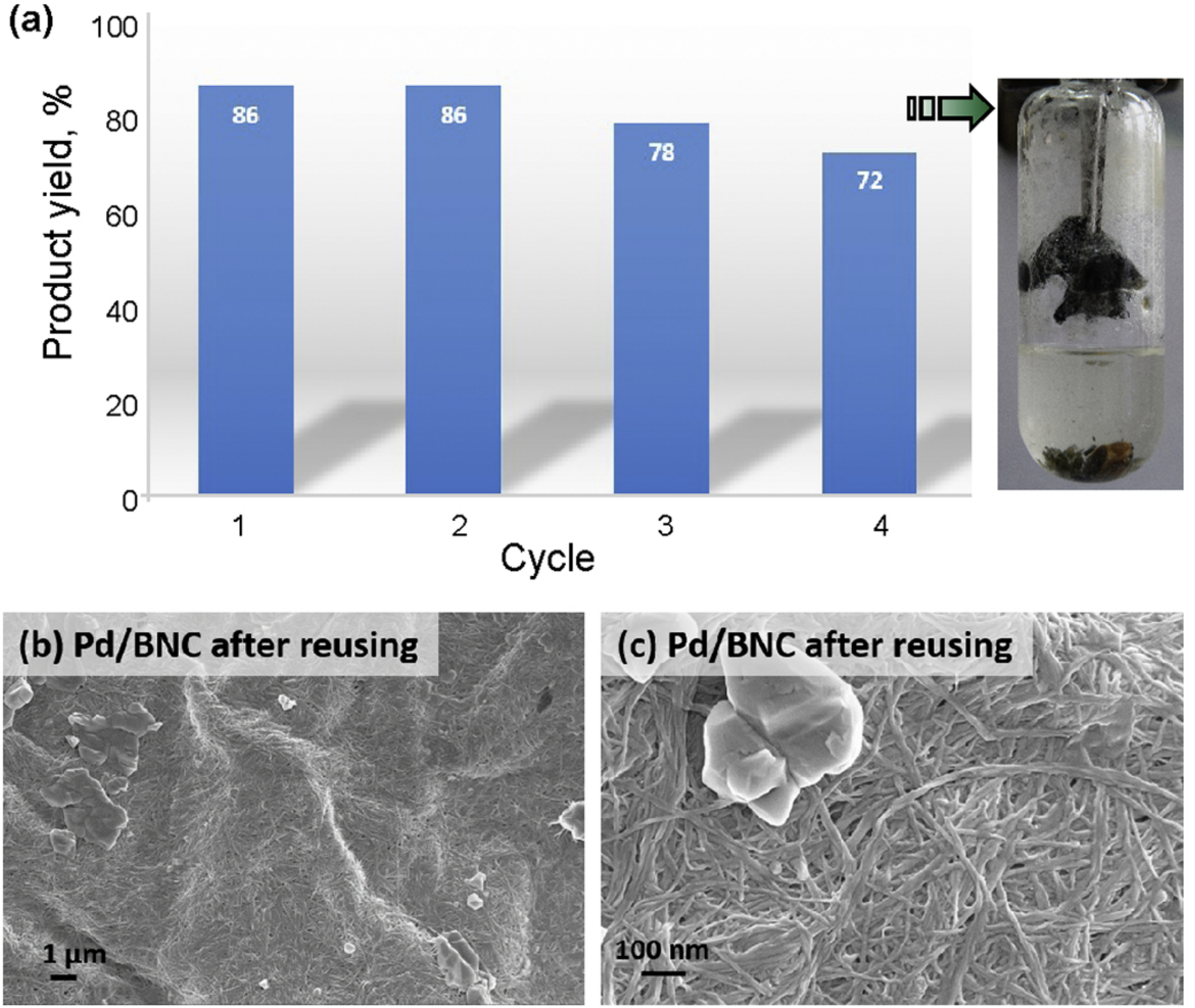

(a)
Product yield, %
100
80
60
40
20
0
86
86
78
72
1
2
3
4
Cycle
(b) Pd/BNC after reusing
1 µm
(c) Pd/BNC after reusing
100 nm

Figure 5

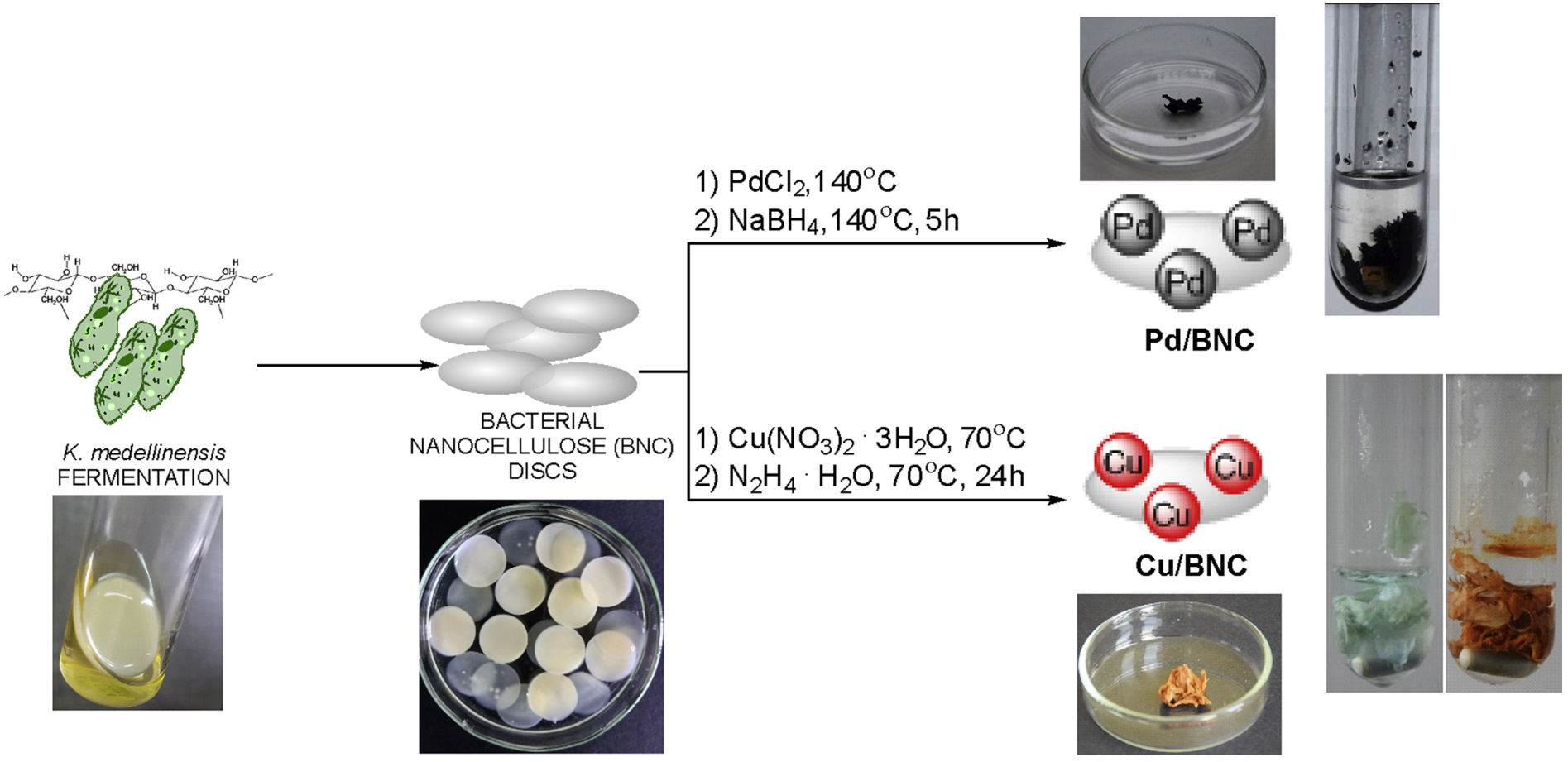

1) $PdCl_2$, 140°C
2) $NaBH_4$, 140°C, 5h
Pd
Pd
Pd
Pd/BNC
BACTERIAL
NANOCELLULOSE (BNC)
DISCS
K. medellinensis
FERMENTATION
1) $Cu(NO_3)_2 \cdot 3H_2O$, 70°C
2) $N_2H_4 \cdot H_2O$, 70°C, 24h
Cu
Cu
Cu
Cu/BNC

Figure 6

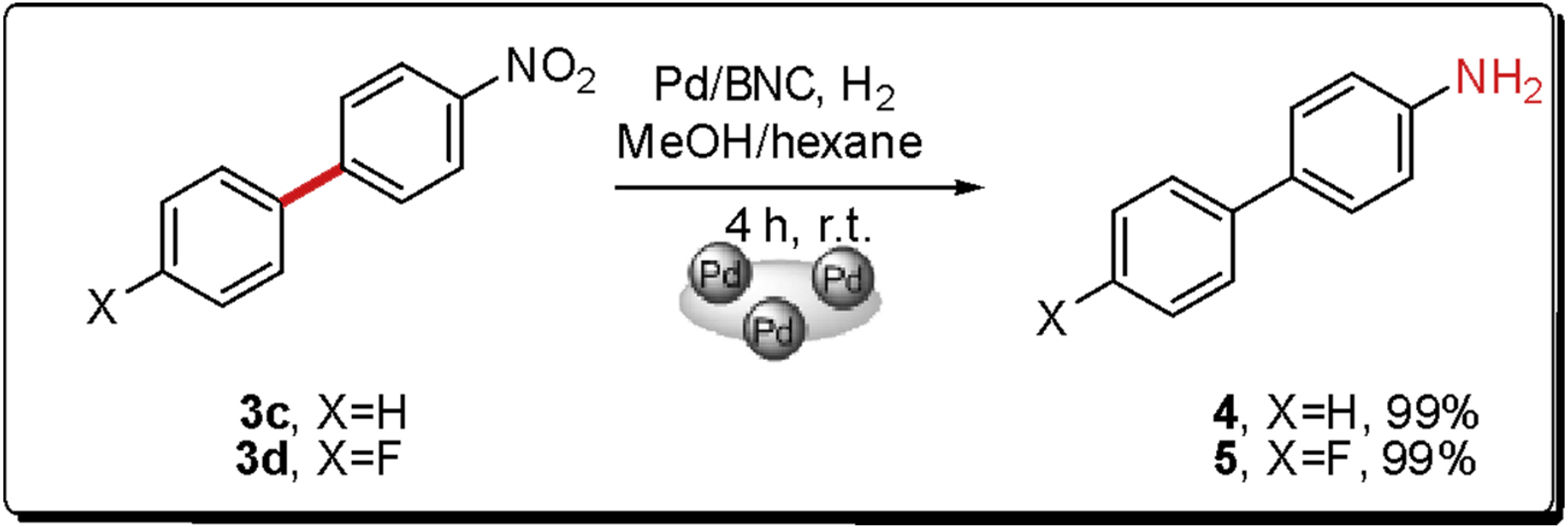

NO2
X
Pd/BNC, H2
MeOH/hexane
4 h, r.t.
Pd
Pd
Pd
NH2
X
3c, X=H
3d, X=F
4, X=H, 99%
5, X=F, 99%

Figure 7

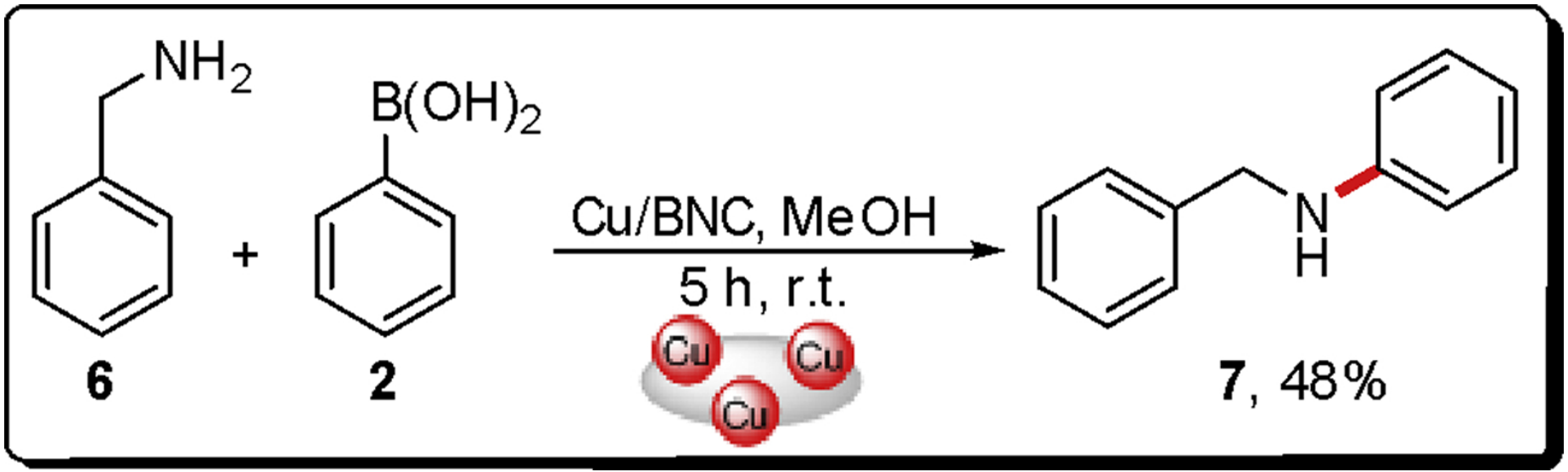



Figure 8