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LAPORAN AKHIR TAHUN
PENELITIAN KOMPETENSI

PENGEMBANGAN PRODUKSI BIOETANOL DALAM
MEDIA LIGNOSELULOSA SEKAM PADI DENGAN KO-KULTUR
Saccharomyces cerevisiae DAN MIKROORGANISME
PENGHASIL BIOETANOL LAIN

TAHUN KE-3 DARI RENCANA 3 TAHUN

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HALAMAN PENGESAHAN

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RINGKASAN

Pemanfaatan materi lignoselulosa dan fermentasi ko-kultur antar mikroorganisme merupakan strategi yang baik untuk meningkatkan produksi etanol dan efisiensi fermentasi. Ko-kultur *Saccharomyces cerevisiae* dengan *Candida tropicalis* dapat memetabolisme dan menkonversi gula sederhana hasil hidrolisis sekam padi menjadi etanol dan mempunyai toleransi terhadap furfural dan phenol. Namun secara umum sekam padi mempunyai kandungan nitrogen yang rendah sehingga perlu disuplementasi nitrogen secara eksternal untuk proses fermentasi. Secara khusus penelitian tahun ketiga bertujuan untuk menemukan kondisi dan nutrisi media fermentasi terbaik untuk produksi etanol dari media sekam padi oleh ko-kultur *S. cerevisiae* dengan *C. tropicalis*. Penelitian ini akan dilakukan secara eksperimental yang terdiri atas beberapa tahap yaitu: (1) menemukan penambahan nitrogen terbaik pada media hidrolisat sekam untuk produksi etanol oleh ko-kultur *S.cerevisiae* dengan *C.tropicalis*, (2) menemukan penambahan proporsi molasses terbaik pada media hidrolisat sekam padi untuk produksi etanol oleh ko-kultur *S.cerevisiae* dengan *C.tropicalis* (3) menemukan pH awal terbaik media hidrolist sekam padi untuk produksi etanol oleh ko-kultur *S.cerevisiae* dengan *C.tropicalis* serta (4) menemukan waktu inkubasi terbaik untuk produksi etanol ko-kultur *S.cerevisiae* dengan *C. tropicalis* dari media hidrolisat sekam padi. Hasil penelitian menunjukkan bahwa sumber nitrogen tidak berpengaruh signifikan ($P>0,05$) terhadap berat kering residu destilat, hasil etanol, serta kadar nitrogen dan karbon residu destilat, namun signifikan ($P<0,05$) dipengaruhi oleh penambahan molasses, formulasi media hidrolisat sekam padi, pH awal media dan waktu inkubasi. Produksi etanol (20.32± 0.42%) dengan kadar nitrogen (4.40± 0.11%) dan karbon (9.20± 1.01%) residu destilat diperoleh dari media hidrolisat sekam padi yang berisi 16.0 g/l urea, 12.0 g/l $NaNO_3$, 12.0 g/l NH_4NO_3, 1.0 g/l KH_2PO_4, 0.7 g/l $MgSO_4 \cdot 7H_2O$, 20 ml/l molasses, 1.0 g/l KH_2PO_4 dan 0.7 g/l $MgSO_4 \cdot 7H_2O$ yang difermentasi pada pH awal media 5.5, waktu inkubasi 5 hari pada suhu 28-29^oC dengan kelembaban relatif 50%, agitasi 60 rpm pada kondisi gelap.

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BAB 1. PENDAHULUAN

Bioetanol berpotensi sebagai bahan bakar cair alternatif pengganti bahan bakar berbasis fosil (minyak bumi) yang persediaannya semakin terbatas (Kongkiattikajorn dan Sornvoraweat, 2011). Biomassa lignoselulosa merupakan bahan baku yang ideal untuk produksi bioetanol sebagai bahan bakar cair yang dapat diperbaharui (Yang, *et al*., 2009). Konversi biomassa lignoselulosa termasuk limbah pertanian menjadi bioetanol merupakan pilihan penting untuk mengeksploitasi sumber energi alternatif dan mengurangi polusi udara (Sa´nchez dan Cardona, 2008; Patel, *et al*, 2012).

Produksi bioetanol pada umumnya dilakukan pada fermentasi monokultur *S.cerevisiae* dalam media karbohidrat khususnya pati (Yuwa-Amornpitak, 2010; Rani *et al*., 2010; Afifi, *et al*., 2011; Misra, *et al*., 2012). Produksi bioetanol di Indonesia mencapai 200.000 kl per tahun menggunakan bahan baku molases, singkong, kentang manis dan jagung (Panaka dan Yudiarto, 2007). Upaya untuk mengembangkan produksi bioetanol dan efisiensi fermentasi pada umumnya dilakukan melalui rekayasa genetik mikroorganisme khususnya *S.cerevisiae.* Namun demikian, upaya pengembangan produksi bioetanol menggunakan mikroorganisme yang telah direkayasa sulit diterapkan masyarakat karena kendala teknologi dan biaya produksi.

Penggunaan materi lignoselulosa sebagai bahan baku produksi bioetanol merupakan salah satu strategi untuk menurunkan biaya produksi dan mengatasi kendala teknologi. Padi merupakan tanaman pangan utama masyarakat Indonesia. Secara kuantitatif sekam padi merupakan produk ikutan yang berpotensi tinggi sebagai bahan baku produk industri termasuk bioetanol. Produksi padi di Indonesia pada tahun 2012 diperkirakan dapat mencapai 68.956.292 ton per tahun (BPS, 2012) dan 20-22% dari produksi padi tersebut berupa sekam padi.

Fermentasi produk hasil hidrolisis lignoselulosa menjadi bioetanol sering kali tidak efisien karena mikroorganisme fermenter tidak dapat mengkonversi semua gula terutama glukosa dan silosa menjadi bioetanol dalam jumlah yang tinggi (Chen, 2011). Gula heksosa dapat difermentasi menjadi bioetanol oleh *S.cerevisiae*, namun hampir tidak ada atau belum ditemukan species khamir dari genus *Saccharomyces* yang mampu mengkonversi gula pentosa seperti silosa menjadi bioetanol (Wan, *et al*., 2012).

Fermentasi ko-kultur merupakan salah satu alternatif untuk produksi bioetanol pada media lignoselulosa. Konsorsium beberapa jenis mikroba menunjukkan aktivitas degradasi yang efisien untuk substrat selulosa termasuk limbah tebu, jerami padi, daun jagung dan limbah industri kertas dari *Eucalyptus* (Wongwilaiwalin, 2010). Fermentasi ko-kultur merupakan strategi yang pada saat ini dikembangkan untuk meningkatkan laju hidrolisis selulosa, memperkaya penggunaan substrat dan meningkatkan hasil produksi melalui kombinasi jalur metabolisme yang berbeda untuk mereduksi efek negatif dari inhibitor (Cheng dan Zhu, 2012). Kokultur *S.cerevisiae* dengan *C. tropicalis* mampu menghasilkan dan mengkonversi gula menjadi etanol (Sopandi dan Wardah, 2015).

Kemampuan khamir untuk memproduksi etanol tergantung pada beberapa faktor seperti strain, faktor pertumbuhan dan kondisi fermentasi (eseperti suhu, laju agitasi, konsentrasi substrat dan inokulum, oksigen dan lain-lain (Dragone *et al*., 2011). Konversi gula hasil hidrolisis lignoselulosik menjadi etanol elemen mikro dan makro dan nitrogen yang mudah difermentasi dalam kesetimbangan yang baik untuk memperoleh hasil yang optimum (Dasqupta, *et al*., 2013). Peningkatan produktivitas dan efektivitas biaya produksi etanol memerlukan beberapa pengembangan proses (Reddy *et al*., 2006; Nuanpeng *et al*., 2011; Khongsay *et al*., 2012; Yue, *et al*., 2012). Efek penambahan berbagai sumber nitrogen seperti ammonium sulfat dan urea pada media dapat meningkatkan pertumbuhan dan produksi etanol (Aziz, *et al*. 2011). Berbagai sumber nitrogen telah digunakan secara luas untuk menstimulasi produksi alkohol seperti ekstrak khamir dan pepton (Bafrncová *et al*. 1999; Bvochorá *et al*. 2000; Reddy dan Reddy, 2006; Laopaiboon *et al*. 2009), ammonium (Laopaiboon *et al*., 2007; Srichuwong *et al*., 2009) dan urea (Yue *et al*., 2010).

BAB 2. TINJAUAN PUSTAKA

Hampir tidak ada atau belum ditemukan mikroorganisme yang mampu memfermentasi semua gula yang dihasilkan dari hidrolisis materi lignoselulosa merupakan masalah utama pemanfaatan lignoselulosa untuk produksi etanol (Zaldivar *et al*., 2001). *Saccharomyces cerevisiae* yang sampai saat ini merupakan khamir dominan untuk produksi etanol secara alami dapat mengkonversi glulosa menjadi etanol

tetapi tidak dapat memetabolisme silosa (Jeffries and Jin, 2004; Lin and Tanakan, 2006).

Selain itu, hidrolisis lignoselulosa untuk menghasilkan gula sederhana juga dapat menghasilkan inhibitor fermentasi (Larsson *et al*., 2001). Hasil etanol dan dan produktivitas yang diperoleh selama fermentasi hidrolisat lignoselulosa menurun karena komponen inhibitor seperti asam lemah, furan dan komponen fenol (Parawira and Tekere, 2011). Komponen inhibitor tersebut dapat menurunkan produksi etanol dan kinerja mikroorganisme selama fermentasi (Almeida *et al*., 2007).

Kajian konsorsium mikroba dan sistem campuran enzim mikroba dapat memberikan dasar-dasar penting untuk memahami interaksi yang komplek dalam proses degradasi lignoselulosa di alam dan dapat dijadikan pijakan dasar untuk aplikasi bioteknologi yang melibatkan degradasi biomassa dalam pembuatan kompos, digesti anaerobik dan sakarifikasi biomass secara enzimatis (Wongwilaiwalin, *et al*., 2010). Kelayakan dari fermentasi ko-kultur untuk produksi bioetanol tergantung pada efisiensi pemanfaatan silosa (Laplace, *et al*., 1993). Fermentasi ko-kultur antar 2 jenis khamir tidak selalu menghasilkan konversi silosa menjadi bioetanol karena prilaku diauksik fermentasi silosa oleh khamir dan kompetisi oksigen antara mikroorganisme fermenter silosa dan fermenter glukosa (deBari, *et al*., 2004; Fu dan Peiris, 2008). Industri fermentasi hidrolisat lignoselulosa menjadi bioetanol memerlukan mikroorganisme yang mempunyai produktivitas bioetanol tinggi dalam berbagai jenis substrat.

Pemilihan mikroorganisme, pemanfaatan substrat secara lengkap, toleransi terhadap inhibitor dan produktivitas merupakan astanol dari materi lignoselulos (Bettiga *et al*., 2009). Efisiensi dan konversi serempak gula pentosa dan heksosa berpengaruh signifikan terhadap pemanfaatan hidrolisat biomassa untuk menghasilkan berbagai produk fermentasi yang bernilai ekonomi (Eiteman *et al.*, 2008).

Fermentasi ko-kultur pada saat ini sedang dikembangkan sebagai sutu strategi untuk meningkatkan laju hidrolisis selulosa, peningkatan pemanfaatan substrat dan meningkatkan produksi etanol melalui kombinasi jalur metabolism untuk mereduksi efek negative inhibitor (Cheng and Zhu, 2012). Penggunaan ko-kultur sebagai suatu proses fermentasi berpotensi jika tidak ada interaksi silang antar mikroorganisme dan

setiap mikroorganisme dapat memetabolisme substrat tanpa dipengaruhi kehadiran mikroorganisme lain (Park *et al*., 2012).

Beberapa hasil penelitian melaporkan bahwa fermentasi ko-kultur *S.cerevisiae* dengan mikroorganisme lain dapat meningkatkan produksi bioetanol. Ko-kultur *S. cerevisiae* dengan *Pachysolen tannmphilus* atau *S.cerevisiae* dengan *Escherichia coli* dapat memproduksi 0,49 g bioetanol per g gula pada media hidrolisat kayu lunak (Qian, *et al*., 2006). Produksi bioetanol pada media hidrolisat jerami gandum dengan ko-kultur *S.cerevisiae* dan *Pichia stipites* lebih tinggi dibandingkan dengan monokultur (Ismail, 2012).

Kebanyakan khamir yang memetabolisme silosa tidak menghasilkan etanol, namun demikian *C. shehatae* dapat menghasilkan etanol (Jeffries dan Alexander, 2005). Fermentasi sekam padi dengan *C. shehatae* dapat menghasilkan etanol 4269 g/l selama 5 hari pada temperatur ruang dengan pH 5,5-6,0. Produksi etanol oleh *C.shehatae* strain ATY839 lebih tinggi dibandingkan *S.cerevisiae* NBRC 0224, *Scheffersomyces stipitis* NBRC 10063, dan *C. shehatae* ATCC 22984 pada media jerami padi yang diberi pelakuan pendahuluan kalium hidroksida (Tanimura, *et al*., 2012). Konsentrasi etanol meningkat 2,6-5,8 dan konsumsi gula meningkat 99 pada fermentasi ko-kultur *C. shehatae* D45-6, *S. cerevisiae* (Cs-Sc), dan *Brettanomyces bruxellensis* dalam media campuran 5% glukosa, 4% silosa dan 5% selobiosa oleh (Sanchez, *et al,*. 2002). Fermentasi ko-kultur *S.cerevisiae* dan *C. tropicalis* dapat memproduksi bioetanol sebanyak 0,35 g/l dalam media sintetis yang mengandung campuran 20 g/l glukosa dan silosa dengan rasio 8:1 sebagai sumber karbon pada suhu inkubasi 30^{o}C selama 18 jam dengan ahitasi 50 rpm (Rodmui, *et al*., 2008).

Ko-kultur *S.cerevisiae* dengan *C. tropicalis* mampu menghasilkan dan mengkonversi gula sederhana dari hasil hidrolisat sekam padi menjadi etanol (Sopandi and Wardah, 2015[a]). Hasil penelitian tersebut dilakukan dengan fermentasi curah menggunakan media sekam padi yang ditambah 4 g/l urea, 3 g/l $NaNO_3$, 3 g/l NH_4NO_3, 1 g/l KH_3PO_4 dan 0.7 g/l $MgSO_4 \cdot 7H_2O$ selama 3 hari pada suhu 30^{o}C, kelembaban relatif 60-70%, keadaan gelap pada agitatasi 150 rpm dapat menghasilkan etanol 2,125±0,259 % dengan efisiensi fermentasi 89.25±10.95%.

Mikroorganisme yang digunakan dalam fermentasi materi lignoselulosa harus toleran terhadap etanol dan senyawa penghambat yang terbentuk selama proses perlakuan pendahuluan (O¨hgren, *et al*., 2011). Furan dan fenol secara umum dapat menghambat pertumbuhan dan laju produksi etanol oleh *S.cerevisiae* (Klinke, *et al*., 2004). Furfural hasil degradasi lignoselulosa tongkol jagung dengan asam sulfat 2,5% merupakan penghambat yang kuat terhadap pertumbuhan serta konsumsi karbon dan nitrogen oleh *P. resticulosum* (Sopandi, *et al*. 2013). Hasil penelitian menunjukkan bahwa ko-kultur *S. cerevisiae* dengan *C. tropicalis* lebih toleran terhadap furfural dan fenol dibandingkan mono kultur *S. cerevisiae* dan *C. tropicalis* (Sopandi dan Wardah., 2015[b])

Nilai pH, sumber nitrogen, nutrisi media, enzim dan ukuran partikel dalam fermentasi ko-kultur berpengaruh terhadap pemanfaatan lignoselulosa dan produksi bioenergi (Cheng dan Zhu, 2012). Penelitian optimasi kondisi dan nutrisi media fermentasi untuk produksi bioetanol oleh fermentasi ko-kultur *S. cerevisiae* dengan *C. tropicalis* dari media sekam padi akan dikerjakan pada penelitian tahun ketiga. Penelitian ini bertujuan menemukan kondisi dan nutrisi media fermentasi cair yang optimum untuk memperoleh produksi etanol dan tingkat efisiensi fermentasi yang maksimum oleh ko-kultur *S. cerevisiae* dengan *C. tropicalis*. Kondisi fermentasi yang dipelajari pada penelitian ini adalah efek penambahan nitrogen dalam media, initial pH media dan waktu inkubasi terhadap produksi bioetanol serta konsumsi karbon dan nitrogen. Penelitian optimasi kondisi dan nutrisi fermentasi cair menggunakan ko-kultur *S. cerevisiae* dengan *C. tropicalis* untuk produksi bioetanol khususnya menggunakan media hidrolisat sekam padi belum pernah dilaporkan. Oleh karena itu, penelitian optimasi kondisi dan nutrisi fermentasi pada fermentasi cair menggunakan ko-kultur *S. cerevisiae* dengan *C. tropicalis* produksi bioetanol dapat menjadi temuan baru.

BAB 3. TUJUAN DAN MANFAAT PENELITIAN

Penelitian tahun ketiga ini bertujuan untuk :

(1) menemukan penambahan nitrogen terbaik pada media hidrolisat sekam untuk produksi etanol oleh ko-kultur *S.cerevisiae* dengan *C.tropicalis*,

(2) menemukan penambahan proposrsi molasses terbaik pada media hidrolisat sekam padi untuk produksi etanol oleh ko-kultur *S.cerevisiae* dengan *C.tropicalis*

(3) menemukan pH awal terbaik media hidrolist sekam padi untuk produksi etanol oleh ko-kultur *S.cerevisiae* dengan *C.tropicalis*

(4) menemukan waktu inkubasi terbaik untuk produksi etanol ko-kultur *S.cerevisiae* dengan *C. tropicalis* dari media hidrolisat sekam padi.

Hasil penelitian ini diduga dapat menghasilkan temuan baru dan diharapkan dapat diaplikasikan untuk produksi bioetanol secara komersial oleh pelaku industri dan masyarakat luas dengan tingkat efisiensi fermentasi yang tinggi.

BAB 4. METODE PENELITIAN

Penelitian tahun ketiga akan akan dilakukan secara eksperimental yang terdiri atas beberapa tahap yaitu:

a. Mikroorganisme dan kondisi biakan

Sebanyak 25 gr media Sabroad dektrose agar dilarutkan dalam 1 l aquadest, dihomogenkan dan disterilisasi pada suhu 121^{o}C selama 15 menit pada tekanan 1 atm. Media selanjutnya dituangkan dalam 50 buah cawan petri masing-masing 20 ml. Setelah dingin dan padat, khamir *Saccharomyces cerevisiae* dan *C. tropicalis* digoreaskan di atas lempengan agar dan diinkubasi pada suhu 28^{o}C selama 7 hari. Peremajaan dan pemeliharaan masing-masing khamir dilakukan secara periodik setiap 3 bulan.

b. Perlakuan pendahuluan sekam padi

Sekam padi dikeringkan, digiling dan dikukus pada suhu 130^{o}C selama 3 jam. Setelah dingin, sebanyak 600 g serbuk sekam padi dihidrolisis 250 ml H_2SO_4 pekat dalam wadah yang berisi 10 l aquadest. Campuran selanjutnya dipanaskan pada suhu 115^{o}C selama 15 menit. Setelah dingin hidrolisat sekam padi disimpan dalam lemari pendingin sampai digunakan.

c. Efek penambahan sumber nitrogen dalam media

Penelitian efek kondisi nutrisi terhadap produksi bioetanol dan efisiensi fermentasi ko-kultur *S. cerevisiae* dengan *C. tropicalis* pada media cair sekam padi akan dikerjakan dengan penambahan nitrogen. Sumber nitrogen yang akan digunakan dalam

penelitian ini terdiri atas urea, $NaNO_3$, dan NH_4NO_3 yang ditambahkan dalam media basal dengan komposisi berbeda, yaitu; 9 g/l urea, 9 g/l $NaNO_3$, dan 9 g/l NH_4NO_3. Komposisi media basal terdiri atas 60 g serbuk sekam padi, 1000 ml aquadest, 1 g/l KH_2PO_4 dan 0.7 g/l $MgSO_4 \cdot 7H_2O$. Sebanyak 1500 ml medium basal dibagi menjadi 3 bagian masing-masing 500 ml dan ke dalam masing-masing bagian ditambahkan 1) 9 g/l urea, 9 g/l $NaNO_3$, dan 9 g/l NH_4NO_3. Campuran dikocok secara sempurna dan masing-masing bagian dibagi lagi menjadi 5 bagian (100 ml) dan dimasukan ke dalam labu Erlenmeyer (250 ml), Nilai pH media diatur dengan menambahkan 0,1% HCl 1 N atau NaOH sampai pH mencapai 5,5. Semua labu Erlenmeyer yang berisi media ditutup dengan kapas dan disterilisasi dalam autoklaf pada suhu 121^oC selama 15 menit. Setelah dingin diinokulasi dengan 10^6/l spora *S. cerevisiae* dan 10^6/l *C. tropicalis*. Semua media yang telah diinokulasi diinkubasi selama 7 hari pada suhu 28-30^oC, kelembaban relatif 60-70% dalam keadaan gelap dengan agitasi 60 rpm. Media yang belum difermentasi dilakukan analisis karbon dan nitrogen. Media yang telah difermentasi dilakukan analisis karbon, nitrogen dan etanol.

d. Efek penambahan molasses

Penelitian efek kondisi nutrisi terhadap produksi bioetanol dan efisiensi fermentasi ko-kultur *S. cerevisiae* dengan *C. tropicalis* pada media cair sekam padi akan dikerjakan dengan penambahan sumber karbon. Sumber karbon yang akan digunakan dalam penelitian ini adalah molases dengan konsentrasi berbeda yang ditambahkan dalam media basal dengan yaitu 0, 5, 10, 15, dan 20 ml/l. Komposisi media basal terdiri atas 60 g serbuk sekam padi, 1000 ml aquadest, 3 g/l urea, 3 g/l $NaNO_3$, 3 g/l NH_4NO, 1 g/l KH_2PO_4 dan 0.7 g/l $MgSO_4 \cdot 7H_2O$. Sebanyak 2500 ml medium basal dibagi menjadi 5 bagian masing-masing 500 ml dan ke dalam masing-masing bagian ditambahkan 0, 5, 10, 15, dan 20 ml/l molasses. Campuran dikocok secara sempurna dan masing-masing bagian dibagi lagi menjadi 5 bagian (100 ml) dan dimasukan ke dalam labu Erlenmeyer (250 ml), Nilai pH media diatur dengan menambahkan 0,1% HCl 1 N atau NaOH sampai pH mencapai 5,5. Semua labu Erlenmeyer yang berisi media ditutup dengan kapas dan disterilisasi dalam autoklaf pada suhu 121^oC selama 15 menit. Setelah dingin diinokulasi dengan 10^6/l spora *S. cerevisiae* dan 10^6/l *C. tropicalis*. Semua media yang telah diinokulasi diinkubasi selama 7 hari pada suhu 28-30^oC, kelembaban relatif 60-

70% dalam keadaan gelap dengan agitasi 60 rpm. Media yang belum difermentasi dilakukan analisis karbon dan nitrogen. Media yang telah difermentasi dilakukan analisis karbon, nitrogen dan etanol.

e. Optimasi penambahan sumber nitrogen dalam media

Optimasi penambahan sumber nitrogen yang akan digunakan dalam penelitian ini terdiri atas urea, $NaNO_3$, dan NH_4NO_3 yang ditambahkan dalam media basal dengan komposisi berbeda, yaitu; 1) 4 g/l urea, 3 g/l $NaNO_3$, 3 g/l NH_4NO_3, 2) 8 g/l urea, 6 g/l $NaNO_3$, 6 g/l NH_4NO_3, 3) 12 g/l urea, 9 g/l $NaNO_3$, 9 g/l NH_4NO_3, dan 4) 16 g/l urea, 12 g/l $NaNO_3$, 12 g/l NH_4NO_3. Komposisi media basal terdiri atas 60 g serbuk sekam padi, 1000 ml hidrolisat sekam padi, 20 ml/l molasses, 1 g/l KH_2PO_4 dan 0.7 g/l $MgSO_4 \cdot 7H_2O$. Sebanyak 2000 ml medium basal dibagi menjadi 4 bagian masing-masing 500 ml dan ke dalam masing-masing bagian ditambahkan 1) 4 g/l urea, 3 g/l $NaNO_3$, 3 g/l NH_4NO_3, 2) 8 g/l urea, 6 g/l $NaNO_3$, 6 g/l NH_4NO_3, 3) 12 g/l urea, 9 g/l $NaNO_3$, 9 g/l NH_4NO_3, dan 4) 16 g/l urea, 12 g/l $NaNO_3$, 12 g/l NH_4NO_3. Campuran dikocok secara sempurna dan masing-masing bagian dibagi lagi menjadi 5 bagian (100 ml) dan dimasukan ke dalam labu Erlenmeyer (250 ml), Nilai pH media diatur dengan menambahkan 0,1% HCl 1 N atau NaOH sampai pH mencapai 5,5. Semua labu Erlenmeyer yang berisi media ditutup dengan kapas dan disterilisasi dalam autoklaf pada suhu 121^oC selama 15 menit. Setelah dingin diinokulasi dengan 10^6/l spora *S. cerevisiae* dan 10^6/l *C. tropicalis*. Semua media yang telah diinokulasi diinkubasi selama 7 hari pada suhu 28-30^oC, kelembaban relatif 60-70% dalam keadaan gelap dengan agitasi 60 rpm. Media yang belum difermentasi dilakukan analisis karbon dan nitrogen. Media yang telah difermentasi dilakukan analisis karbon, nitrogen dan etanol.

f. Efek pH awal media

Sebanyak 4000 ml media hidrolisat sekam padi yang mengandung 20 ml/l molasses, 12 g/l urea, 9 g/l $NaNO_3$, 9 g/l NH_4NO_3, 1 g/l KH_2PO_4 dan 0.7 g/l $MgSO_4 \cdot 7H_2O$ dibagi menjadi 8 bagian masing-masing 250 ml dan masing-masing bagian ditambahkan 0,1% HCl 1 N atau NaOH untuk memperoleh pH awal media 3,0, 3,5, 4,0, 4,5, 5,0, 5,5, 6,5, 7,0. Selanjutnya masing-masing bagian dibagi lagi menjadi 5 bagian (100 ml) dan dimasukan ke dalam labu Erlenmeyer (250 ml). Semua labu Erlenmeyer yang berisi media ditutup dengan kapas dan disterilisasi dalam autoklaf

pada suhu 121^{o}C selama 15 menit. Setelah dingin diinokulasi dengan 10^6/l spora 10^6/l *S. cerevisiae* 10^6/l *C. tropicalis* dan diinkubasi selama 7 hari pada suhu 28-30^{o}C, kelembaban relatif 60-70% dalam keadaan gelap dengan agitasi 60 rpm. Media yang telah difermentasi dilakukan analisis karbon dan nitrogen dan bioetanol.

g. Efek waktu inkubasi

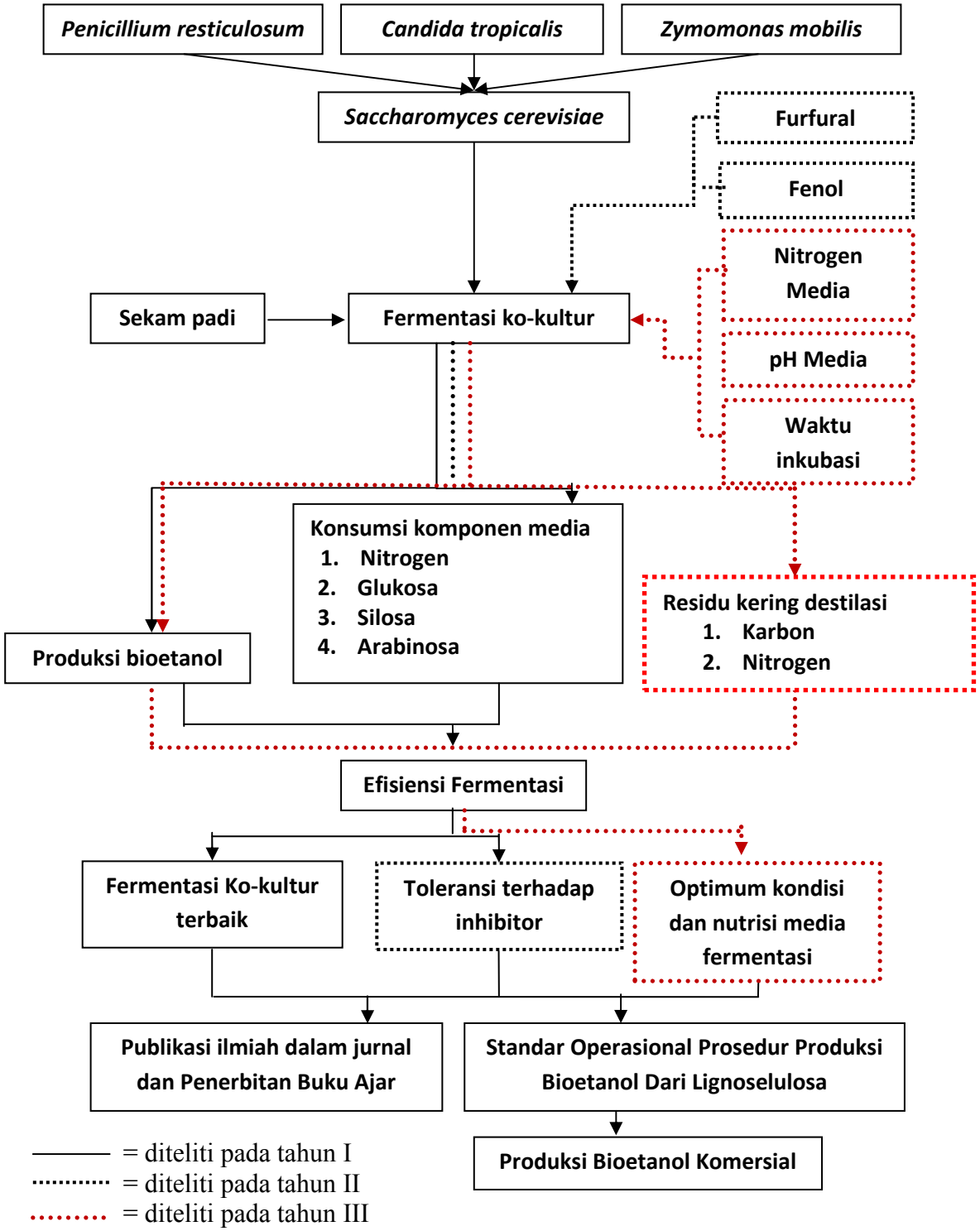
Sebanyak 5000 ml media hidrolisat sekam padi yang mengandung 12 g/l urea, 9 g/l $NaNO_3$, 9 g/l NH_4NO_3, 1 g/l KH_2PO_4 dan 0.7 g/l $MgSO_4 \cdot 7H_2O$. Campuran dikocok secara sempurna dan masing-masing bagian dibagi lagi menjadi 10 bagian (100 ml) dan dimasukan ke dalam labu Erlenmeyer (250 ml), Nilai pH media diatur dengan menambahkan 0,1% HCl 1 N atau NaOH sampai pH mencapai 5,5. Setelah dingin diinokulasi dengan 10^6/l spora 10^6/l *S. cerevisiae* 10^6/l *C. tropicalis* dan diinkubasi dengan waktu yang berbeda pada suhu 28-30^{o}C, kelembaban relatif 60-70% dalam keadaan gelap dengan agitasi 60 rpm. Waktu inkubasi yang digunakan terdiri atas 0, 1, 2, 3, 4, 5, 6, 7, 8 dan 9 hari. Media yang telah difermentasi dilakukan analisis karbon dan nitrogen dan bioetanol.

h. Analisis bioetanol

Media cair sebelum dan setelah difermentasi dipanen dan dimasukan ke dalam labu Erlenmeyer melalui kertas saring Whatman No. 1. Analisis bioetanol dilakukan dengan kromatografi gas menggunakan detektor ionisasi nyala dengan nitrogen sebagai gas pembawa, kemasan kolom baja Porapack Q dengan panjang 100 cm, ukuran mesh 80-100, suhu injektor dan ditektor 200^{o}C dan suhu kolom 180^{o}C. Isopropanol digunakan sebagai standar internal.

i. Analisis karbon

Kadar karbon organik ditentukan dengan menggunakan metode oksidasi basah Walkey and Black (1965). Sebanyak 50 ml kultur cair dievaporasi pada 100^{o}C selama 2 jam dan dikeringkan pada suhu 60^{o}C sampai berat konstan untuk memperoleh serbuk kering. Sampel sebanyak 0.5 g digunakan untuk determinasi TOC yang diukur pada panjang gelombang 651 nm. Sebanyak 0,50 g serbuk kering residu destilasi ukuran mess < 0,5 mm, dimasukkan ke dalam labu ukur 100 ml, ditambahkan 5 ml K2Cr2O7 1 N dan dikocok. Setelah tercampur ditambahkan 7,5 ml H2SO4 pekat, dikocok lalu diamkan selama 30 menit. Diencerkan dengan air bebas ion, biarkan dingin dan



Gambar 4.1. Bagan alir pencapaian tujuan akhir penelitian

diimpitkan selama 12 jam. Absorbansi larutan jernih diukur dengan spektrofotometer pada panjang gelombang 561 nm. Sebagai pembanding dibuat standar karbon dengan konsentrasi 0 dan 250 ppm, dengan memipet 0 dan 5 ml larutan standar 5.000 ppm ke dalam labu ukur 100 ml dengan perlakuan yang sama dengan pengerjaan sampel serbuk destilasi kering.

j. Analisis nitrogen

Konsentrasi (NH_4-N) ditentukan menggunakan metoda the American Society of Agronomy and Soil Science Society of America (1982). Sebanyak 10 ml kultur media dievaporasi pada suhu 100^oC selama 2 jam untuk memperoleh serbuk kering. Sampel (0.5 g) ditambahkan ke dalam tabung digesti dan ditambahkan 1 g campuran selenium (1.55 g Kristal $CuSO_4$, 96.9 g Na_2SO_4 dan 1.55 g selenium) serta ditambahkan 3 ml 97% H_2SO_4, dicampur dan didigesti pada suhu 350ºC selama 4 jam untuk memperoleh ektrak tak berwarna, dinginkan pada suhu ruang, diencerkan dengan 50 ml air distilat, kocok dan diamkan satu malam. Dua ml ekstrak dipindahkan ke dalam tabung gelas borosilikat baru tambahkan secara berurutan 4 ml tartrate buffer (50 g NaOH dan 50 g $KNaC_4H_4O_6$ /l) dan larutan sodium phenate (100 g NaOH dan 125 g phenol/l), kocok dan biarkan selama 10 min. Sebanyak 4 ml, 5% NaOCl ditambahkan, kocok dan biarkan selama 10 min. Selanjutnya absorbansi diukur pada panjang gelombang 636 nm. $(NH_4)_2SO_4$ digunakan sebagai standar nitrogen.

j. Efisiensi fermentasi

Efisiensi fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* untuk produksi etanol kedua media sekam padi dan tongkol jagung menggunakan formula efisiensi fermentasi yang diuraikan pada tahun pertama.

k.Analisis statistika

Data hasil pengamatan akan dianalisis menggunakan analisis sidik ragam (ONOVA) taraf 0,05 dan jika terdapat pengaruh yang signifikan ($P<0,05$) untuk mengetahui letak perbedaan dilakukan uji lanjut dengan uji beda nyata jujur pada taraf kepercayaan 0,05.

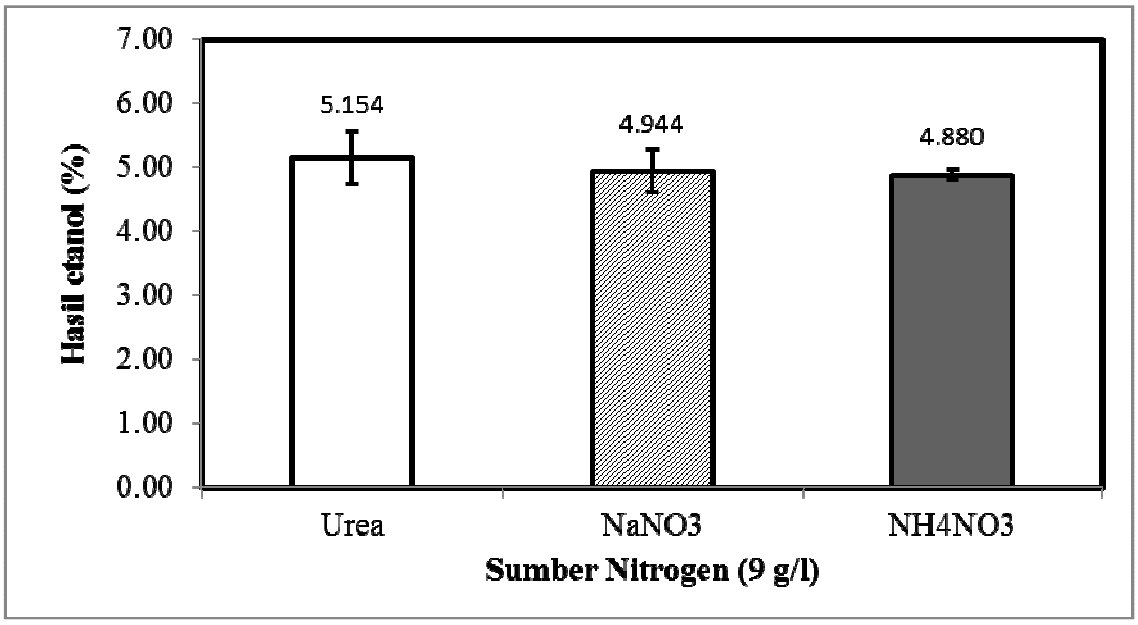
BAB 5. HASIL DAN LUARAN YANG DICAPAI

5.1 Hasil Penelitian

5.1.1. Efek penambahan nitrogen dalam media

a. **Hasil etanol**

Hasil penelitian (Gambar 5.1.1) menunjukkan bahwa hasil etanol dari media hidrolisat sekam padi yang difermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* tidak dipengaruhi secara signifikan (P>0.05) oleh penambahan 3 jenis sumber nitrogen. Hasil etanol dari media hidrolisat sekam padi yang ditambah 9 g/l urea dan difermentasi oleh ko-kultur *S. cerevisiae* dan *C. tropicalis* (5,154± 0,416%) tidak berbeda signifikan (P>0,05) dengan hasil etanol dari media hidrolisat sekam padi yang ditambah 9 g/l $NaNO_3$ (4,944± 0,342%) dan hasil etanol dari media hidrolisat sekam padi yang ditambah 9 g/l NH_4NO_3 (4,880± 0,077%).

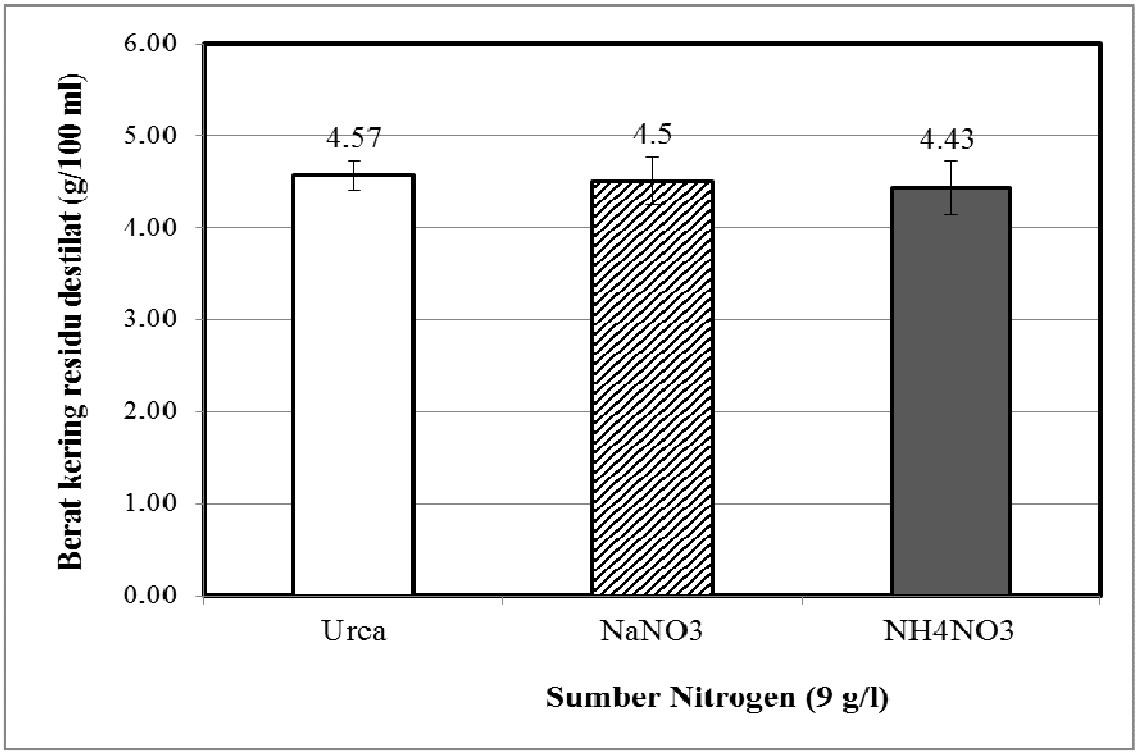


Gambar 5.1.1. Hasil etanol dari media sekam padi yang ditambah sumber nitrogen dan difermentasi oleh ko-kultur *S. cerevisiae* dan *C. tropicalis*.

b. **Bobot residu destilat**

Hasil penelitian berat residu kering destilat media hidrolisat sekam padi yang ditambah sumber nitrogen dan difermentasi dengan ko-kultur *S. cerevisiae* dan *C. tropicalis* disajikan pada Gambar 5.1.2. Hasil penelitian memperlihatkan bahwa jenis

sumber nitrogen tidak berpengaruh signifikan (P>0.05) terhadap berat kering residu destilat media yang telah difermentasi dengan ko-kultur *S. cerevisiae* dan *C. tropicalis*.

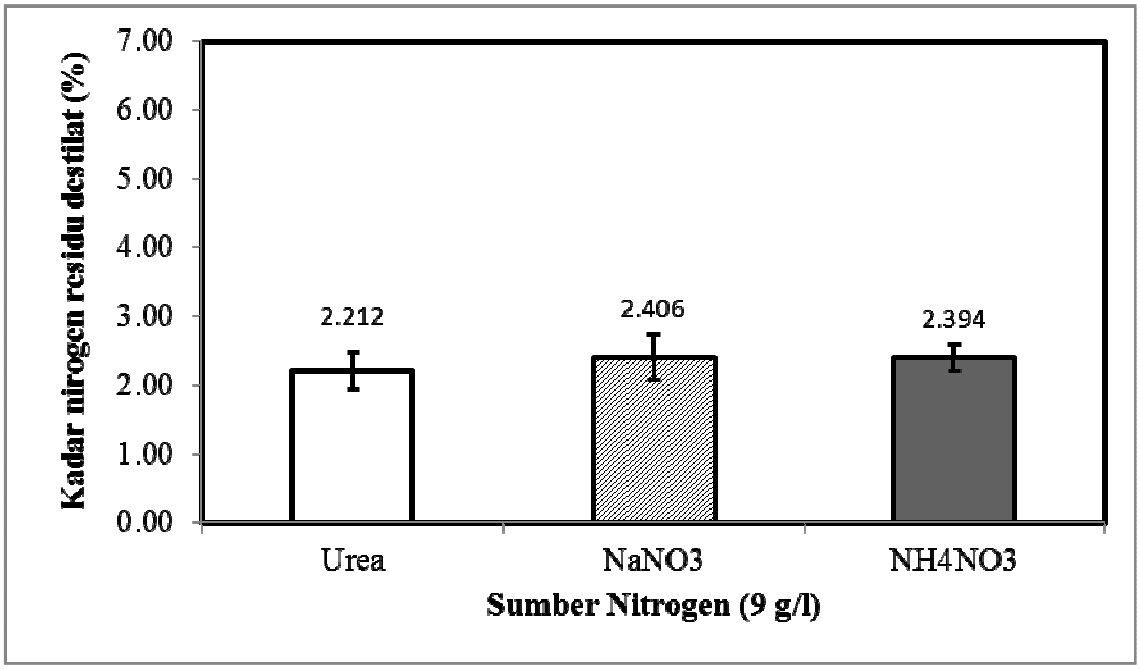


Gambar 5.1.2. Bobot residu kering destilat media sekam padi yang ditambah sumber nitrogen dan difermentasi dengan ko-kultur *S. cerevisiae* dan *C. tropicalis*.

c. Kadar nitrogen residu destilat

Hasil penelitian kadar nitrogen residu kering destilat media hidrolisat sekam padi yang ditambah 3 jenis sumber nitrogen yang berbeda dan difermentasi dengan ko-kultur *S. cerevisiae* dan *C. tropicalis* disajikan pada Gambar 5.1.3. Hasil penelitian memperlihatkan bahwa jenis sumber nitrogen tidak berpengaruh signifikan (P>0,05) terhadap kadar nitrogen residu destilat media hidrolisat sekam padi yang telah difermentasi oleh ko-kultur *S. cerevisiae* dan *C. tropicalis*. Kadar nitrogen residu destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* dari media hidrolisat sekam padi yang ditambah 9 g/l urea (2,212± 0.265%) tidak berbeda signifikan (P>0,05) dengan kadar nitrogen residu destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C.*

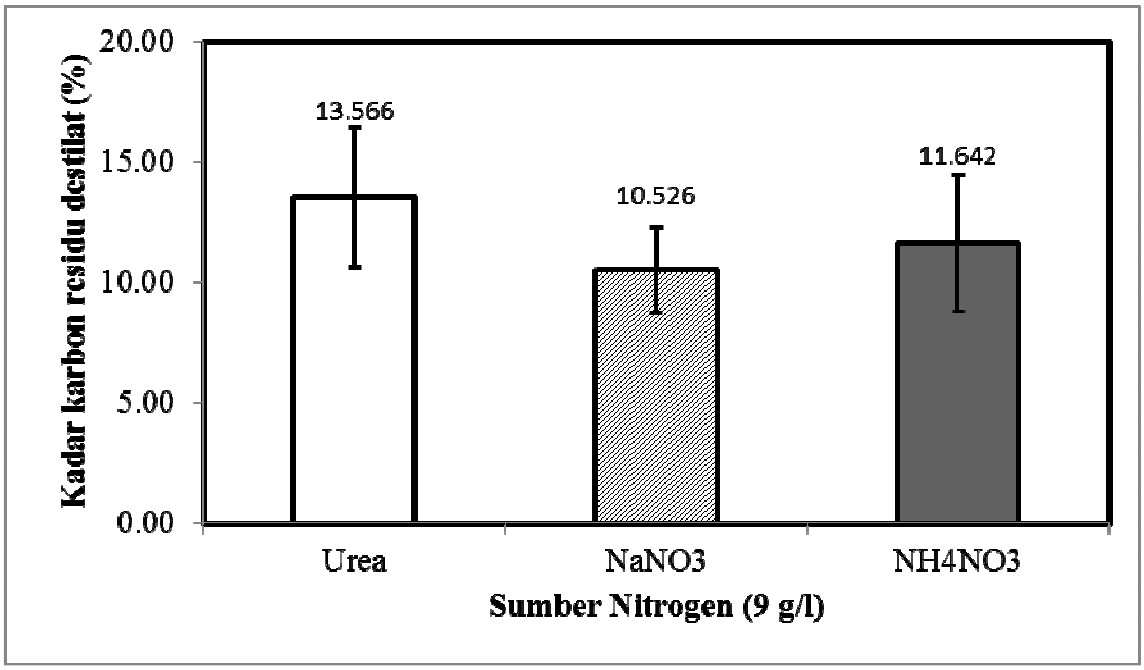
tropicalis dari media hidrolisat sekam padi yang ditambah 9 g/l $NaNO_3$ (2,406± 0,341%) dan dari media yang ditambah NH_4NO_3 (2,394± 0,199%).



Gambar 5.1.3. Kadar nitrogen residu kering destilat media sekam padi yang ditambah sumber nitrogen dan difermentasi dengan ko-kultur *S. cerevisiae* dan *C. tropicalis*.

d. Kadar karbon residu destilat

Hasil penelitian kadar karbon residu kering destilat media hidrolisat sekam padi yang ditambah 3 jenis sumber nitrogen yang berbeda dan difermentasi dengan ko-kultur *S. cerevisiae* dan *C. tropicalis* disajikan pada Gambar 5.1.3. Hasil penelitian memperlihatkan bahwa jenis sumber nitrogen tidak berpengaruh signifikan (P>0,05) terhadap kadar karbon residu destilat media hidrolisat sekam padi yang telah difermentasi oleh ko-kultur *S. cerevisiae* dan *C. tropicalis*. Kadar karbon residu destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* dari media hidrolisat sekam padi yang ditambah 9 g/l urea (13,566± 2,897%) tidak berbeda signifikan (P>0,05) dengan kadar karbon residu destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* dari media hidrolisat sekam padi yang ditambah 9 g/l $NaNO_3$ (10,526± 1,786%) dan dari media yang ditambah NH_4NO_3 (11,642± 2,829%).



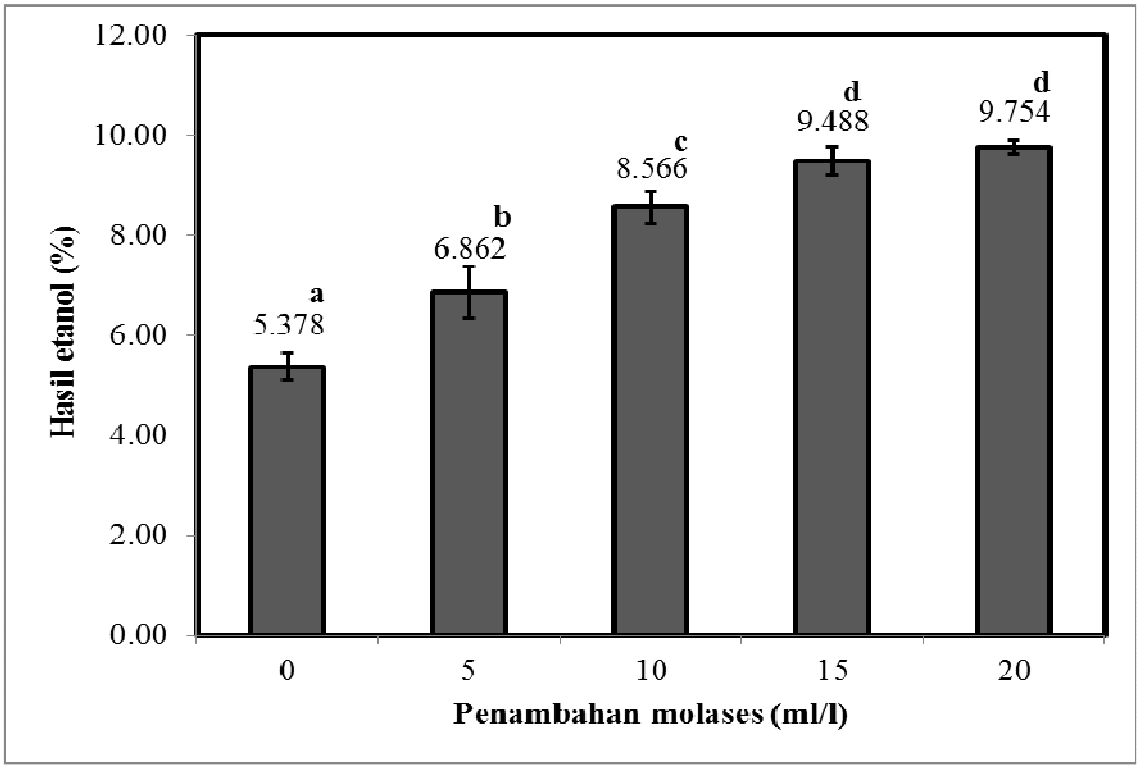
Gambar 5.1.4. Kadar karbon residu kering destilat media sekam padi yang ditambah sumber nitrogen dan difermentasi dengan ko-kultur *S. cerevisiae* dan *C. tropicalis*.

5.1.2 **Efek penambahan molases dalam media**

a. Hasil etanol

Hasil penelitian (Gambar 5.1.5) menunjukkan bahwa hasil etanol dari media hidrolisat sekam padi yang difermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* dipengaruhi secara signifikan (P<0.05) oleh penambahan proporsi molases. Hasil etanol dari media hidrolisat sekam padi yang ditambah 0,0 ml/l molasses signifikan (P<0,05) lebih rendah dibandingkan dengan hasil etanol dari media hidrolisat sekam padi yang ditambah 5,0 ml/l molasses (6,862± 0,522%), 10 ml/l (8,566± 0,313%), 15 ml/l (9,488± 0,287%) dan 20 ml/l molasses (9,754± 0,139%). Hasil etanol dari media hidrolisat sekam padi yang ditambah molasses 5,0 ml/l (6,862± 0,522%) signifikan (P<0,05) lebih rendah dibandingkan dengan hasil etanol dari media hidrolisat sekam padi yang ditambah 10 ml/l (8,566± 0,313%), 15 ml/l (9,488± 0,287%) dan 20 ml/l molasses (9,754± 0,139%). Hasil etanol dari media hidrolisat sekam padi yang ditambah 10 ml/l molasses (8,566± 0,313%) signifikan (P<0,05) lebih rendah dibandingkan dengan hasil etanol dari media hidrolisat sekam padi yang ditambah 15 ml/l (9,488± 0,287%) dan 20 ml/l molasses (9,754± 0,139%). Namun tidak terdapat perbedaan yang signifikan

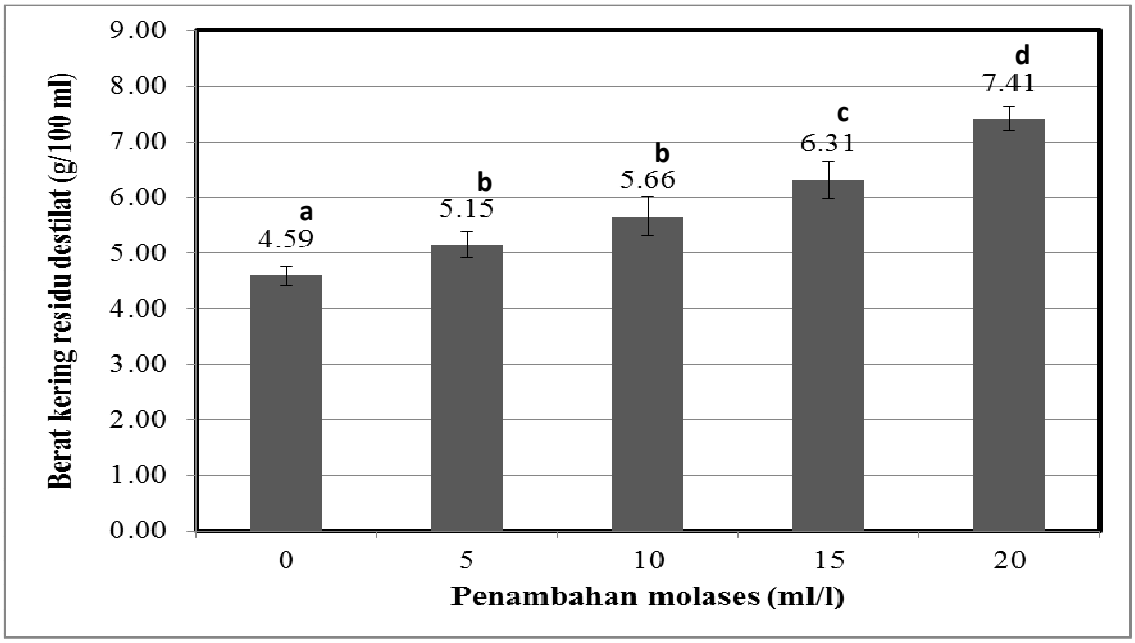
(P>0,05) antara hasil etanol dari media hidrolisat sekam padi yang ditambah 15 ml/l (9,488± 0,287%) dengan hasil etanol dari media hidrolisat sekam padi yang ditambah 20 ml/l (9,754± 0,139%).



Gambar 5.1.5. Hasil etanol dari media sekam padi yang ditambah molasses dengan proporsi yang berbeda dan difermentasi oleh ko-kultur *S. cerevisiae* dan *C. tropicalis*.

b. Berat kering residu destilat

Hasil penelitian berat kering residu destilat media sekam padi yang ditambah molasses sebagai sumber karbon dan difermentasi dengan ko-kultur *S. cerevisiae* dan *C. tropicalis* disajikan pada Gambar 5.1.6. Hasil penelitian memperlihatkan bahwa penambahan molasses dalam media berpengaruh signifikan (P<0,05) terhadap berat kering residu destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis*. Berat kering residu destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* pada media yang tidak ditambah molasses (4,59±0,17 g/100 ml)) signifikan (P<0,05) lebih rendah dibandingkan dengan berat kering residu destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* pada media yang ditambah 5 (5,15±0,23 g/100 ml), 10 (5,66±0,35 g/100 ml), 15 (6,31±0,33 g/100 ml) dan 20 (7,40±0,21 g/100 ml) ml/l.

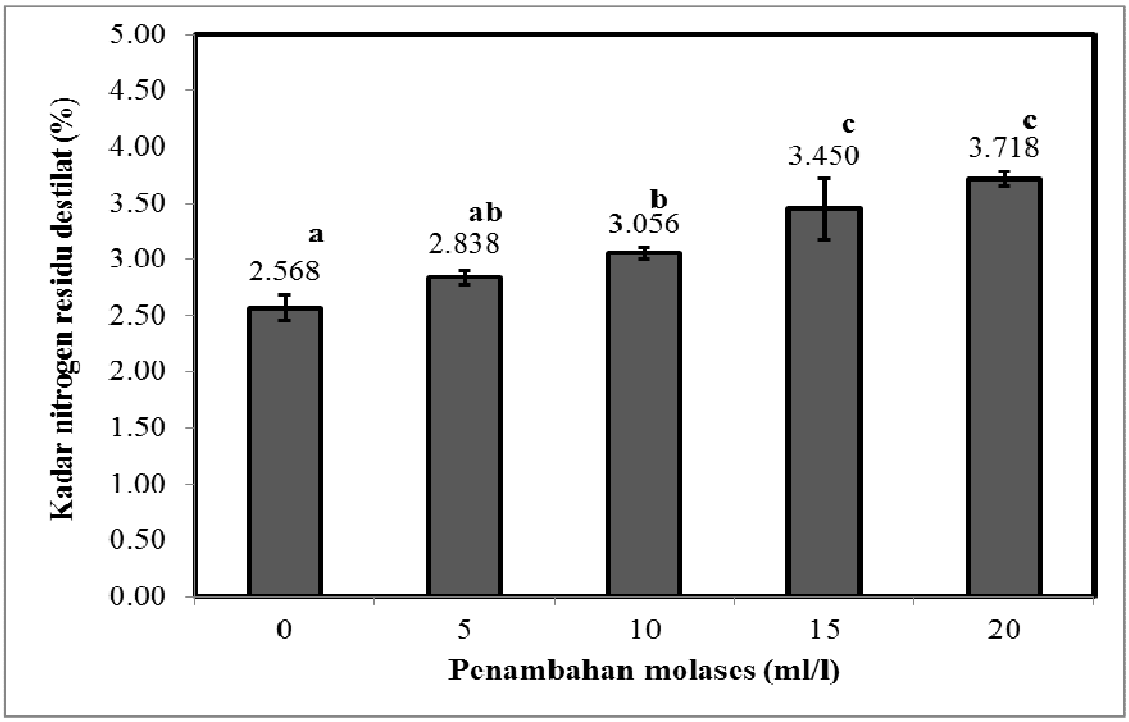


Gambar 5.1.6. Berat residu kering destilat media hidrolisat sekam padi yang ditambah molases dan difermentasi dengan ko-kultur *S. cerevisiae* dan *C. tropicalis*.

c. Kadar nitrogen residu destilat

Hasil penelitian kadar nitrogen residu destilat media sekam padi yang ditambah molasses sebagai sumber karbon dan difermentasi dengan ko-kultur *S. cerevisiae* dan *C. tropicalis* disajikan pada Gambar 5.1.7. Hasil penelitian memperlihatkan bahwa penambahan molasses dalam media hidrolisat sekam padi berpengaruh signifikan (P<0,05) terhadap kadar nitrogen residu destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis.* Kadar nitrogen residu destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* pada media hidrolisat sekam padi yang tidak ditambah molasses (2,568± 0,115 %) tidak berbeda signifikan (P>0,05) dengan kadar nitrogen residu destilat media hidrolisat sekam padi yang ditambah 5 ml/l molasses (2,838±0,065%), namun signifikan (P<0,05) lebih rendah dibandingkan dengan kadar nitrogen residu destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* pada media yang ditambah 10 ml/l (3,056±0,053%), 15 ml/l (3,450 ±0,276%) dan 20 ml/l (3,718±0,063%). Kadar nitrogen residu destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* pada media hidrolisat sekam padi yang ditambah molasses 5 ml/l (2,838±0,065%) tidak berbeda signifikan (P>0,05) dengan kadar nitrogen residu destilat

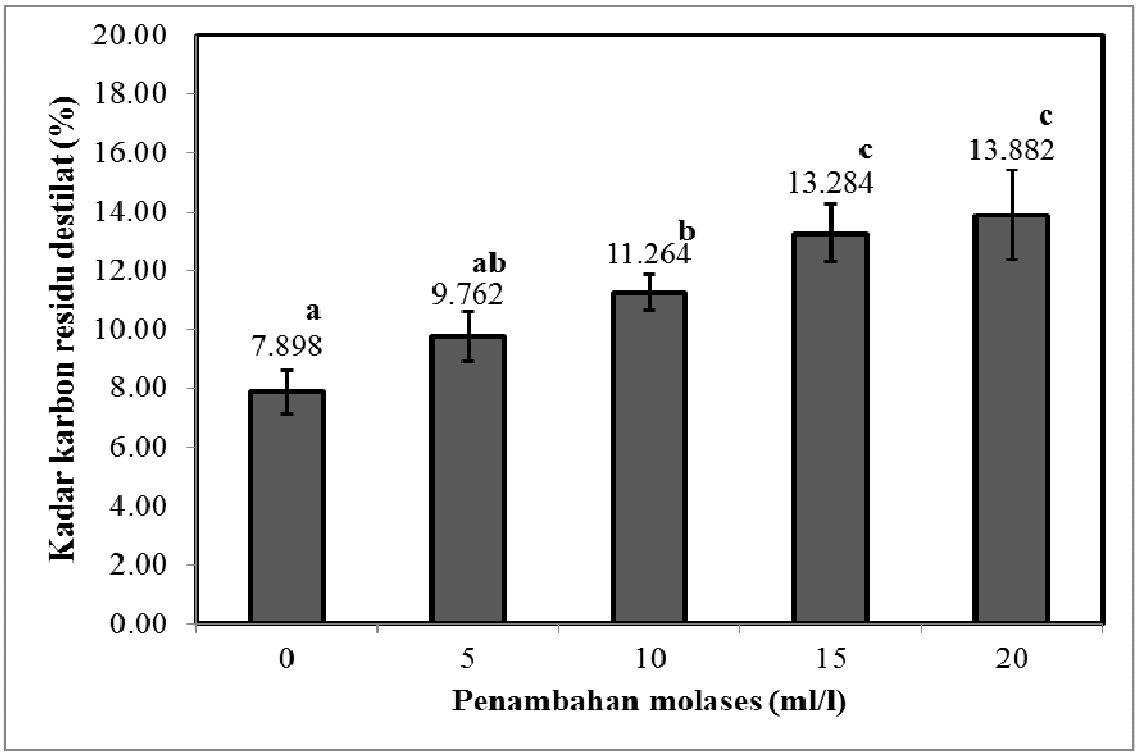
media hidrolisat sekam padi yang ditambah molasses 10 ml/l (3,056±0,053%), namun signifikan (P<0,05) lebih rendah dibandingkan dengan kadar nitrogen residu destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* pada media yang ditambah molasses 15 ml/l (3,450 ±0,276%) dan 20 ml/l (3,718±0,063%). Kadar nitrogen residu destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* pada media hidrolisat sekam padi yang ditambah molasses 10 ml/l (3.056±0.053%) signifikan (P<0,05) lebih rendah dibandingkan dengan kadar nitrogen residu destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* pada media yang ditambah molasses 15 ml/l (3,450 ±0,276%) dan 20 ml/l (3,718±0,063%). Kadar nitrogen residu destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* pada media hidrolisat sekam padi yang ditambah molasses 15 ml/l (3,450 ±0,276%) tidak berbeda signifikan (P>0,05) dengan kadar nitrogen residu destilat media hidrolisat sekam padi yang ditambah molasses 20 ml/l (3,718±0,063%).



Gambar 5.1.7. Kadar nitrogen residu destilat dari media hidrolisat sekam padi yang ditambah molases dan difermentasi dengan ko-kultur *S. cerevisiae* dan *C. tropicalis*.

d. Kadar karbon residu destilat

Hasil penelitian kadar karbon residu destilat media sekam padi yang ditambah molasses sebagai sumber karbon dan difermentasi dengan ko-kultur *S. cerevisiae* dan *C. tropicalis* disajikan pada Gambar 5.1.8. Hasil penelitian memperlihatkan bahwa penambahan molasses dalam media hidrolisat sekam padi berpengaruh signifikan (P<0,05) terhadap kadar karbon residu destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis*. Kadar karbon residu destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* pada media hidrolisat sekam padi yang tidak ditambah molasses (7,898±0,729%) tidak berbeda signifikan (P>0,05) dengan kadar karbon residu destilat media hidrolisat sekam padi yang ditambah 5 ml/l molasses (9,762± 0,846%), namun signifikan (P<0,05) lebih rendah dibandingkan dengan kadar karbon residu destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* pada media yang ditambah molasses 10 ml/l (11,264± 0,602%), 15 ml/l (13,284± 0,985%) dan 20 ml/l (13,882± 1,521%). Kadar karbon residu destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* pada media hidrolisat sekam padi yang ditambah molasses 5 ml/l (9,762± 0,846%) tidak berbeda signifikan (P>0,05) dengan kadar nitrogen residu destilat media hidrolisat sekam padi yang ditambah molasses 10 ml/l (11,264± 0,602%), namun signifikan (P<0,05) lebih rendah dibandingkan dengan kadar nitrogen residu destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* pada media yang ditambah molasses 15 ml/l (13,284± 0,985%) dan 20 ml/l (13,882± 1,521%). Kadar karbon residu destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* pada media hidrolisat sekam padi yang ditambah molasses 10 ml/l (11,264± 0,602%) signifikan (P<0,05) lebih rendah dibandingkan dengan kadar nitrogen residu destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* pada media yang ditambah molasses 15 ml/l (13,284± 0,985%) dan 20 ml/l (13,882± 1,521%). Kadar karbon residu destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* pada media hidrolisat sekam padi yang ditambah molasses 15 ml/l 15 ml/l (13,284± 0,985%) tidak berbeda signifikan (P>0,05) dengan kadar nitrogen residu destilat media hidrolisat sekam padi yang ditambah molasses 20 ml/l (13,882± 1,521%).



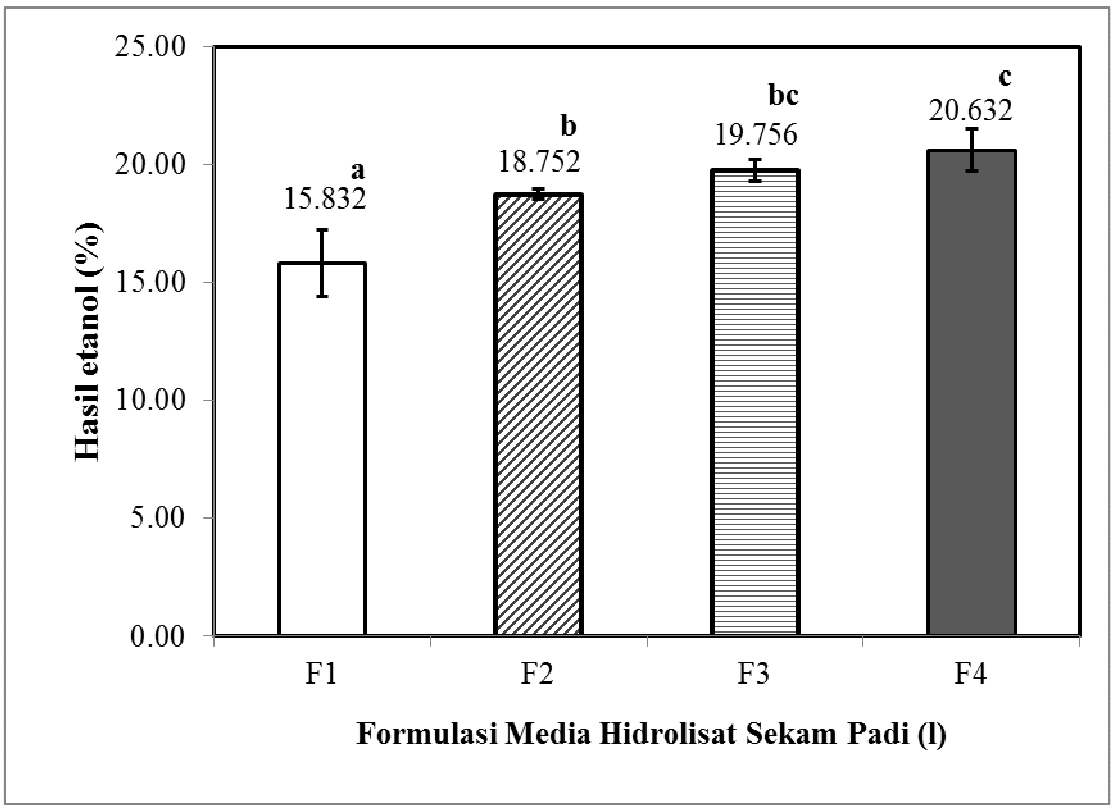
Gambar 5.1.8. Kadar karbon residu destilat dari media hidrolisat sekam padi yang ditambah molases dan difermentasi dengan ko-kultur *S. cerevisiae* dan *C. tropicalis*.

5.1.3 Formulasi media hidrolisat sekam padi

a. Hasil etanol

Hasil penelitian memperlihatkan bahwa 4 macam media formulasi hidrolisat sekam padi yang disuplementasi dengan nitrogen dan molasses berpengaruh signifikan ($P<0,05$) terhadap etanol hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis*. Gambar 5.1.9 menunjukkan bahwa hasil etanol dari fermentasi media formulasi F1 (4 g/l urea, 3 g/l $NaNO_3$, 3 g/l NH_4NO_3, 20 ml/l molasses) yaitu 15,832± 1,429% signifikan ($P<0,05$) lebih rendah dibandingkan dengan hasil etanol dari fermentasi media formulasi F2 (8 g/l urea, 6 g/l $NaNO_3$, 6 g/l NH_4NO_3, 20 ml/l molasses) yaitu 18,752± 0,229%, F3 (12 g/l urea, 9 g/l $NaNO_3$, 9 g/l NH_4NO_3, 20 ml/l molasses) yaitu 19,756±0,467% dan F4 (16 g/l urea, 12 g/l $NaNO_3$, 12 g/l NH_4NO_3, 20 ml/l molasses yaitu 20,632± 0,890%. Hasil etanol dari fermentasi media formulasi F2 (18,752±0,229%) tidak berbeda signifikan ($P>0,05$) dibandingkan dengan hasil etanol

dari fermentasi media formulasi F3 (19,756±0,467%), namun signifikan (P<0,05) dibandingkan dengan hasil etanol dari fermentasi media formulasi F4 (20,632±0,890%). Hasil etanol dari fermentasi media formulasi F3 (19,756±0,467%) tidak berbeda signifikan (P>0,05) dibandingkan hasil etanol dari fermentasi media formulasi F4 (20,632±0,890%).



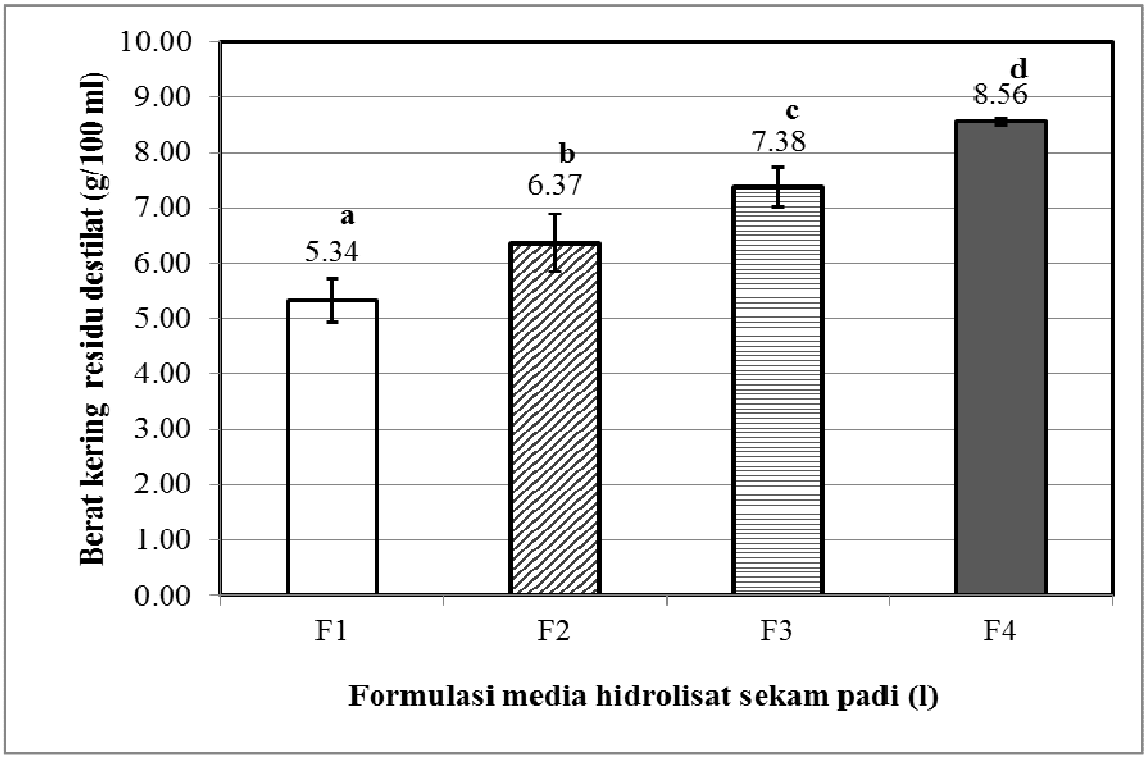
Keterangan : F1= 4 g/l urea, 3 g/l $NaNO_3$, 3 g/l NH_4NO_3, 20 ml/l molasses; F2= 8 g/l urea, 6 g/l $NaNO_3$, 6 g/l NH_4NO_3, 20 ml/l molasses; F3= 12 g/l urea, 9 g/l $NaNO_3$, 9 g/l NH_4NO_3, 20 ml/l molasses; F4 = 16 g/l urea, 12 g/l $NaNO_3$, 12 g/l NH_4NO_3, 20 ml/l molasses

Gambar 5.1.9. Hasil etanol dari fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* 4 formulasi media hidrolisat sekam padi.

b. Berat residu destilat

Hasil penelitian berat residu kering destilat dari 4 macam formulasi media hidrolisat sekam padi yang difermentasi dengan ko-kultur *S. cerevisiae* dan *C. tropicalis* disajikan pada Gambar 5.1.10. Hasil penelitian memperlihatkan bahwa rata-rata berat

kering residu destilat setelah difermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis*.signifikan (P<0,05) berbeda antar formulasi media hidrolisat sekam padi.



Keterangan : F1= 4 g/l urea, 3 g/l $NaNO_3$, 3 g/l NH_4NO_3, 20 ml/l molasses; F2= 8 g/l urea, 6 g/l $NaNO_3$, 6 g/l NH_4NO_3, 20 ml/l molasses; F3= 12 g/l urea, 9 g/l $NaNO_3$, 9 g/l NH_4NO_3, 20 ml/l molasses; F4 = 16 g/l urea, 12 g/l $NaNO_3$, 12 g/l NH_4NO_3, 20 ml/l molasses

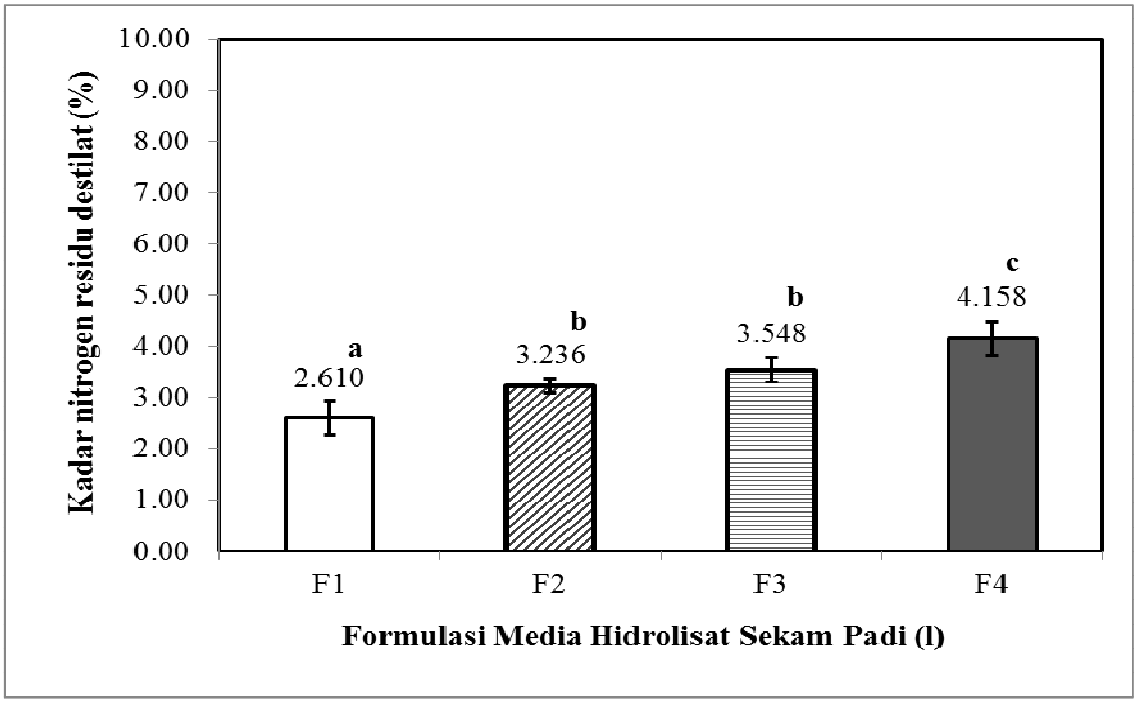
Gambar 5.1.10. Berat residu kering destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* dari 4 formulasi media hidrolisat sekam padi.

Berat kering residu destilat dari hasil fermentasi media formulasi F1 (4 g/l urea, 3 g/l $NaNO_3$, 3 g/l NH_4NO_3, 10 ml/l molasses) yaitu 5,34±0,39 g/100 ml signifikan (P<0,05) lebih rendah dibandingkan dengan berat kering residu destilat dari hasil fermentasi media formulasi F2 (8 g/l urea, 6 g/l $NaNO_3$, 6 g/l NH_4NO_3,20 ml/l molasses yaitu 6,37±0,52 g/100 ml), F3 (12 g/l urea, 9 g/l $NaNO_3$, 9 g/l NH_4NO_3, 20 ml/l molasses yaitu 7,38±0,36 g/100 ml dan F4 (16 g/l urea, 12 g/l $NaNO_3$, 12 g/l NH_4NO_3, 20 ml/l molasses yaitu 8,56±0,06 g/100 ml. Berat kering residu destilat dari hasil fermentasi media formulasi F2 (6,37±0,52 g/100 ml) signifikan (P<0,05) lebih rendah dibandingkan dengan berat kering residu destilat dari hasil fermentasi media formulasi

F3 (7,38±0,36 g/100 ml) dan F4 (8,56±0,06 g/100 ml). Berat kering residu destilat dari hasil fermentasi media formulasi F3 (7,38±0,36 g/100 ml) signifikan (P<0,05) lebih rendah dibandingkan dengan berat kering residu destilat dari hasil fermentasi media formulasi F4 (8,56±0,06 g/100 ml).

c. Kadar nitrogen residu destilat

Hasil penelitian kadar nitrogen residu kering destilat dari 4 macam formulasi media hidrolisat sekam padi yang difermentasi dengan ko-kultur *S. cerevisiae* dan *C. tropicalis* disajikan pada Gambar 5.1.11. Hasil penelitian memperlihatkan bahwa rata-rata kadar nitrogen residu kering destilat setelah difermentasi *S. cerevisiae* dan *C. tropicalis* berbeda signifikan (P<0,05) antar formulasi media hidrolisat sekam padi.



Keterangan : F1= 4 g/l urea, 3 g/l $NaNO_3$, 3 g/l NH_4NO_3, 20 ml/l molasses; F2= 8 g/l urea, 6 g/l $NaNO_3$, 6 g/l NH_4NO_3, 20 ml/l molasses; F3= 12 g/l urea, 9 g/l $NaNO_3$, 9 g/l NH_4NO_3, 20 ml/l molasses; F4 = 16 g/l urea, 12 g/l $NaNO_3$, 12 g/l NH_4NO_3, 20 ml/l molasses

Gambar 5.1.11. Kadar nitrogen residu kering destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* dari 4 formulasi media hidrolisat sekam padi.

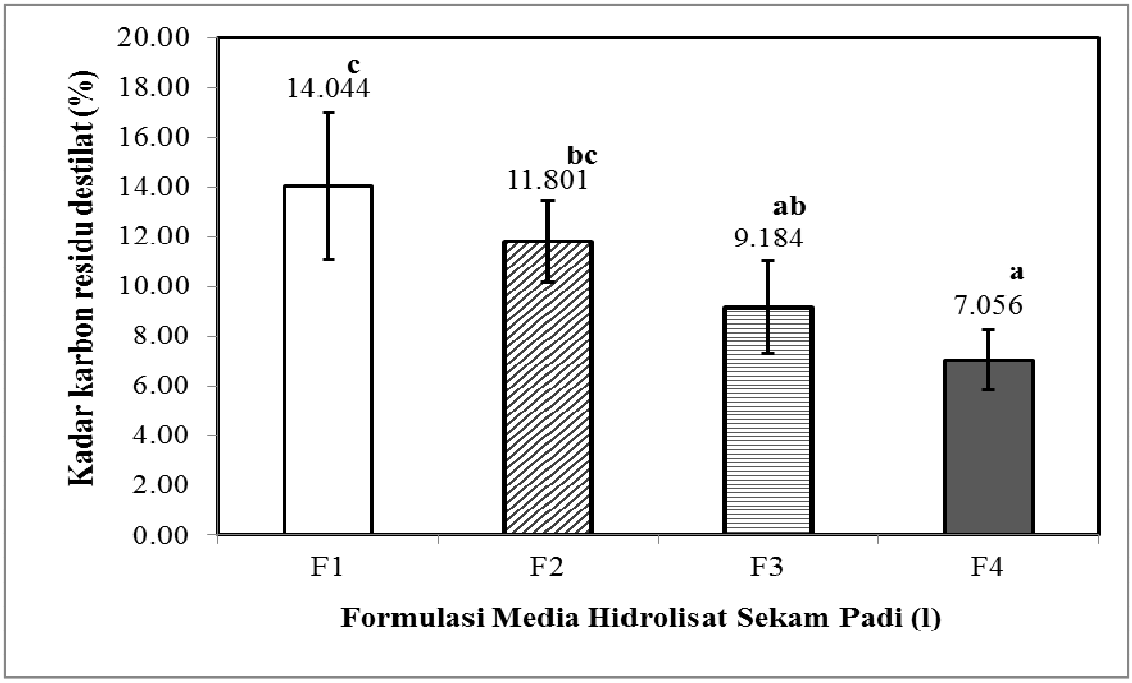
Rata-rata kadar nitrogen residu destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* formulasi media F1 (4 g/l urea, 3 g/l $NaNO_3$, 3 g/l NH_4NO_3, 20 ml/l molasses) yaitu 2,610±0,343% signifikan (P<0,05) lebih rendah dibandingkan dengan rata-rata kadar nitrogen residu destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* formulasi media F2 (8 g/l urea, 6 g/l $NaNO_3$, 6 g/l NH_4NO_3, 20 ml/l molasses) yaitu 3,236±0,147%, F3 (12 g/l urea, 9 g/l $NaNO_3$, 9 g/l NH_4NO_3, 20 ml/l molasses) yaitu 3,548±0,235%, dan F4 (16 g/l urea, 12 g/l $NaNO_3$, 12 g/l NH_4NO_3, 20 ml/l molasses) yaitu 4,158±0,319%. Rata-rata kadar nitrogen residu destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* formulasi media F2 (3,236±0,147%) tidak berbeda signifikan (P>0,05) dengan rata-rata kadar nitrogen residu destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* formulasi media F3 (3,548 ± 0,235%), namun signifikan (P<0,05) lebih rendah dibandingkan dengan rata-rata kadar nitrogen residu destilat hasil fermentasi fomulasi media F4 (4,158±0,319%). Rata-rata kadar nitrogen residu destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* formulasi media F3 (3,548 ± 0,235%) signifikan (P<0,05) lebih rendah dibandingkan dengan rata-rata kadar nitrogen residu destilat hasil fermentasi fomulasi media F4 (4,158±0,319%).

d. Kadar karbon residu destilat

Hasil penelitian kadar karbon residu kering destilat dari 4 macam formulasi media hidrolisat sekam padi yang difermentasi dengan ko-kultur *S. cerevisiae* dan *C. tropicalis* disajikan pada Gambar 5.1.12. Hasil penelitian memperlihatkan bahwa rata-rata kadar karbon residu kering destilat setelah difermentasi *S. cerevisiae* dan *C. tropicalis* berbeda signifikan (P<0,05) antar formulasi media hidrolisat sekam padi.

Rata-rata kadar karbon residu destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* formulasi media F1 (4 g/l urea, 3 g/l $NaNO_3$, 3 g/l NH_4NO_3, 20 ml/l molasses) yaitu 14,044± 2,965% tidak berbeda signifikan (P>0,05) dibandingkan dengan rata-rata kadar karbon residu destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* formulasi media F2 (8 g/l urea, 6 g/l $NaNO_3$, 6 g/l NH_4NO_3, 20 ml/l molasses) yaitu 11,801± 1,629%, namun signifikan (P<0,05) lebih tinggi dibandingkan dengan F3 (12 g/l urea, 9 g/l $NaNO_3$, 9 g/l NH_4NO_3, 20 ml/l molasses) yaitu 9.184±

1,883%, dan F4 (16 g/l urea, 12 g/l $NaNO_3$, 12 g/l NH_4NO_3, 20 ml/l molasses) yaitu 7,056± 1,209%. Rata-rata kadar karbon residu destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* formulasi media F2 (11,801± 1,629%) tidak berbeda signifikan (P>0,05) dengan rata-rata kadar karbon residu destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* formulasi media F3 (9.184± 1,883%), namun signifikan (P<0,05) lebih tinggi dibandingkan dengan rata-rata kadar karbon residu destilat hasil fermentasi fomulasi media F4 (7,056± 1,209%). Rata-rata kadar karbon residu destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* formulasi media F3 (9.184± 1,883%) tidak berbeda signifikan (P>0,05) dibandingkan dengan rata-rata kadar karbon residu destilat hasil fermentasi fomulasi media F4 (7,056± 1,209%).



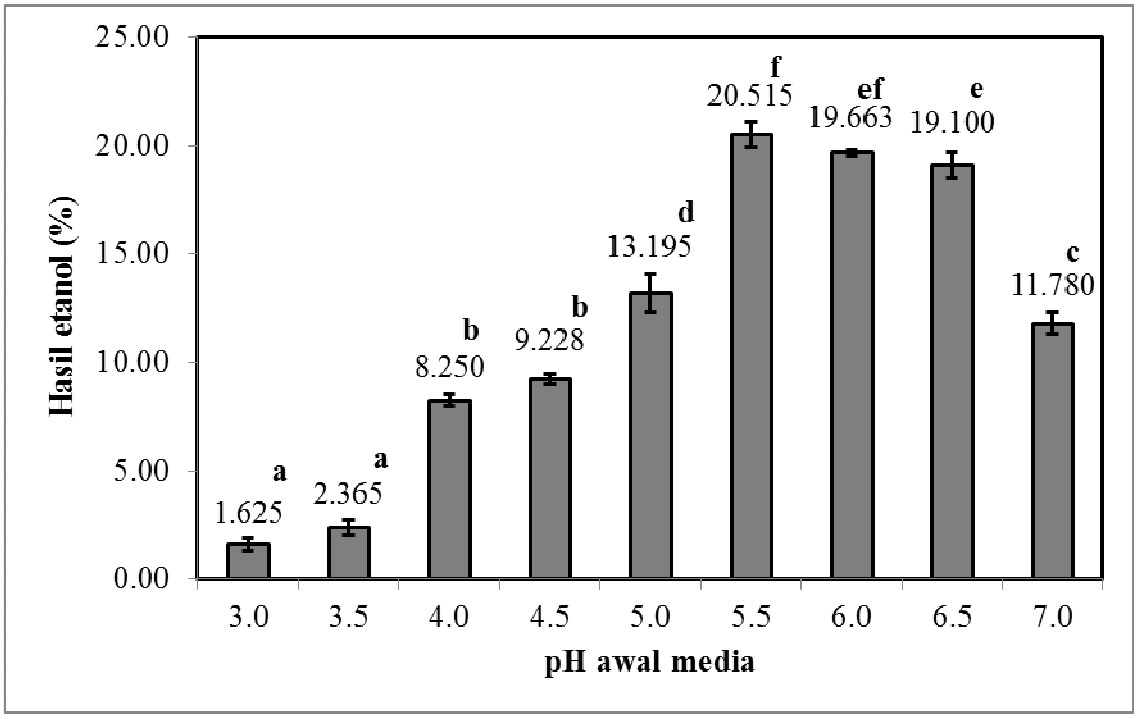
Keterangan : F1= 4 g/l urea, 3 g/l $NaNO_3$, 3 g/l NH_4NO_3, 20 ml/l molasses; F2= 8 g/l urea, 6 g/l $NaNO_3$, 6 g/l NH_4NO_3, 20 ml/l molasses; F3= 12 g/l urea, 9 g/l $NaNO_3$, 9 g/l NH_4NO_3, 20 ml/l molasses; F4 = 16 g/l urea, 12 g/l $NaNO_3$, 12 g/l NH_4NO_3, 20 ml/l molasses

Gambar 5.1.12. Kadar karbon residu kering destilat hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* dari 4 formulasi media hidrolisat sekam padi.

5.1.4 **Efek pH awal dalam media**

a. Hasil etanol

Hasil etanol dari fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* media formulasi media sekam padi pada pH awal yang berbeda disajikan pada Gambar 5.1.13. Hasil penelitian memperlihatkan bahwa pH awal media berpengaruh signifikan (P<0.05) terhadap hasil etanol fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* dari formulasi hidrolisat sekam padi.



Gambar 5.1.3. Hasil etanol fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* formulasi hidrolisat sekam padi pada pH awal yang berbeda

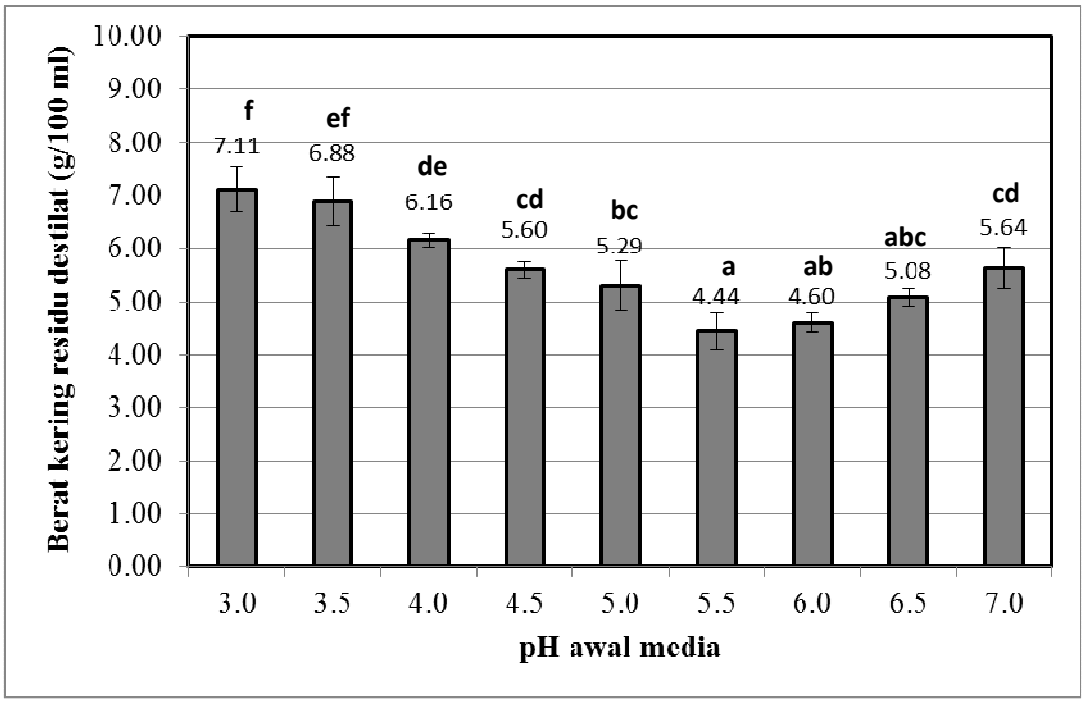
Penelitian memperlihatkan bahwa hasil etanol pada pH awal media 3,0 (1,625 ± 0,309%) tidak berbeda signifikan (P>0,05) dibandingkan hasil etanol pada pH awal media 3,5 (2,365± 0,357%), namun signifikan lebih rendah dibandingkan dengan hasil etanol pada pH 4,0 (8,250± 0,263%), 4,5 (9,228± 0,259%), 5,0 (13,195± 0,888%), 5,5 (20,515± 0,602%), 6,0 (19,663± 0,155%), 6,5 (19,100± 0,584%) dan pH 7,0 (11,780±0,501%). Rata-rata hasil etanol pada pH awal media 4,0 (8,250± 0,263%) tidak berbeda signifikan (P>0,05) dibandingkan dengan rata-rata hasil etanol pada pH awal media 4,5 (9,228± 0,259%), namun signifikan (P<0,05) lebih rendah dibandingkan hasil etanol pada pH awal media 5,0 (13,195± 0,888%), 5,5 (20,515± 0,602%), 6,0 (19,663±

0,155%), 6,5 (19,100± 0,584%) dan pH 7,0 (11,780±0,501%). Rata-rata hasil etanol pada pH awal media 5,0 (13,195± 0,888%) signifikan (P<0,05) lebih rendah dibandingkan hasil etanol pada pH awal media 5,5 (20,515± 0,602%), 6,0 (19,663± 0,155%), dan pH 6,5 (19,100±0,584%), namun signifikan (P<0,05) lebih tinggi dibandingkan dengan pH awal media 7,0 (6,780±0,501%). Rata-rata hasil etanol pada pH awal media 5,5 (20,515± 0,602%) tidak berbeda signifikan (P>0,05) dibandingkan dengan hasil etanol pada pH awal media 6,0 (19,663± 0,155%), namun signifikan lebih tinggi dibandingkan hasil etanol pada pH awal media 6,5 (19,100±0,584%).

b. Berat kering residu destilat

Hasil penelitian berat residu kering destilat media hidrolisat sekam padi yang difermentasi dengan ko-kultur *S. cerevisiae* dan *C. tropicalis* pada pH awal yang berbeda disajikan pada Gambar 5.1.4. pH awal media berpengaruh signifikan (P<0.05) terhadap berat kering residu destilat media yang telah difementasi dengan ko-kultur *S. cerevisiae* dan *C. tropicalis*.

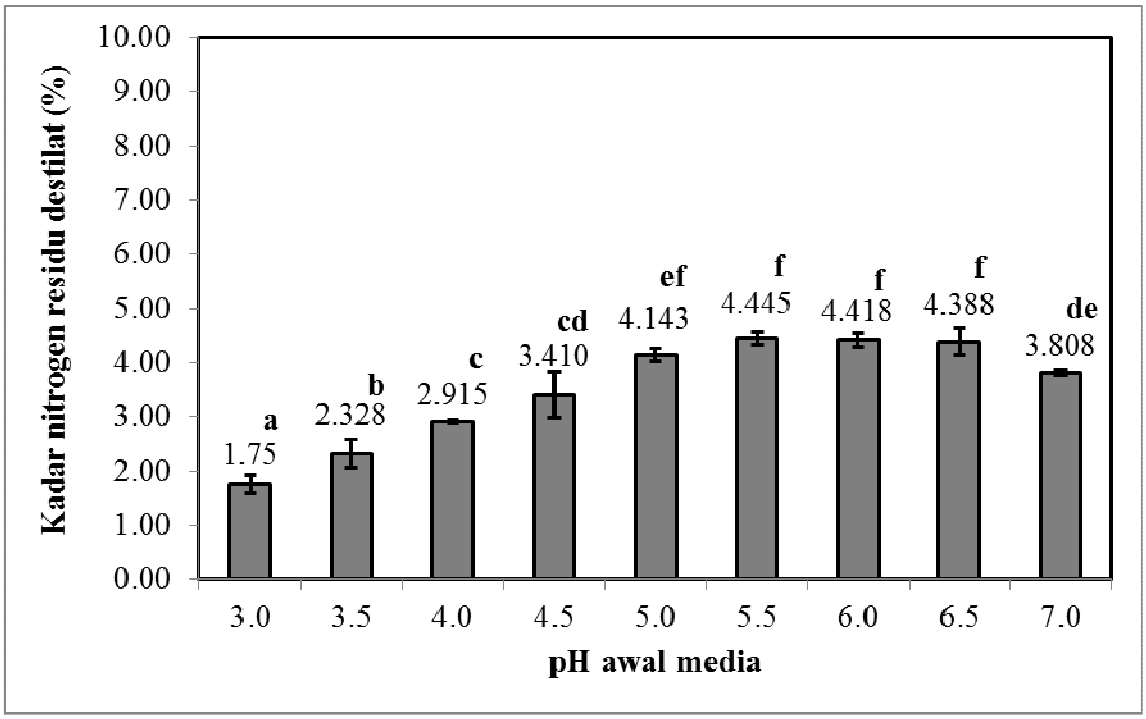
Hasil penelitian memperlihatkan bahwa berat kering residu destilat pada pH awal media 5,5 (4,44±0,34 g/100 ml) signifikan (P<0,05) lebih rendah dibandingkan berat kering residu destilat pada pH awal media 5,0 (5,29±0,47 g/100 ml), 4,5 (5,60±0,15 g/100 ml), 7 (5,65±0,39 g/100 ml), 4,0 (6,16±0,13 g/100 ml), 3,5 (6,88±0,46 g/100 ml), dan 3,0 (7,10±0,42 g/100 ml), namun tidak berbeda signifikan (P>0,05) dibandingkan dengan berat kering residu destilat pada pH 6,0 (4,60±0,17 g/100 ml) dan pH 6,5 (5,08±0,16 g/100 ml). Berat kering residu destilat pada pH awal media 6,0 (4,60±0,17 g/100 ml) signifikan (P<0,05) lebih rendah dibandingkan berat kering residu destilat pada pH awal media 4,5 (5,60±0,15 g/100 ml), 7 (5,65±0,39 g/100 ml), 4,0 (6,16±0,13 g/100 ml), 3,5 (6,88±0,46 g/100 ml), dan 3,0 (7,10±0,42 g/100 ml), namun tidak berbeda signifikan (P>0,05) dibandingkan dengan berat kering residu destilat pada pH 6,5 (5,08±0,16 g/100 ml). Berat kering residu destilat pada pH awal media 6,5 (5,08±0,16 g/100 ml) signifikan (P<0,05) lebih rendah dibandingkan berat kering residu destilat pada pH awal media 4,5 (5,60±0,15 g/100 ml), 7 (5,65±0,39 g/100 ml), 4,0 (6,16±0,13 g/100 ml), 3,5 (6,88±0,46 g/100 ml), dan 3,0 (7,10±0,42 g/100 ml).



Gambar 5.1.4. Berat residu kering destilat media hidrolisat sekam padi yang difermentasi dengan ko-kultur *S. cerevisiae* dan *C. tropicalis* pada pH awal yang berbeda

c. Kadar nitrogen residu destilat

Hasil penelitian kadar nitrogen residu destilat media hidrolisat sekam padi yang difermentasi dengan ko-kultur *S. cerevisiae* dan *C. tropicalis* pada pH awal yang berbeda disajikan pada Gambar 5.1.5. Kadar nitrogen residu destilat media hidrolisat sekam padi hasil fementasi ko-kultur *S. cerevisiae* dan *C. tropicalis* signifikan ($P<0,05$) dipengaruhi oleh pH awal media.



Gambar 5.1.5. Kadar nitrogen residu kering destilat media hidrolisat sekam padi yang difermentasi dengan ko-kultur *S. cerevisiae* dan *C. tropicalis* pada pH awal yang berbeda

Hasil penelitian memperlihatkan bahwa rata-rata kadar nitrogen residu destilat pada pH awal media 3,0 (1,750±0,166%) signifikan (P<0,05) lebih rendah dibandingkan dengan rata-rata kadar nitrogen residu destilat pada pH 3,5 (2,328± 0,261%), pH 4,0 (2,915± 0,033%), pH 4,5 (3,410±0,424%), pH 5,0 (4,143±0,121%), pH 5,5 (4,445±0,122%), pH 6,0 (4,418±0,132%), pH 6,5 (4,388±0,258%), dan pH 7,0 (3,808±0,054%). Rata-rata kadar nitrogen residu destilat pada pH awal media pH 3,5 (2,328± 0,261%) signifikan (P<0,05) lebih rendah dibandingkan dengan rata-rata kadar nitrogen residu destilat pada pH awal media 4,0 (2,915± 0,033%), pH 4,5 (3,410±0,424%), pH 5,0 (4,143±0,121%), pH 5,5 (4,445±0,122%), pH 6,0 (4,418±0,132%), pH 6,5 (4,388±0,258%), dan pH 7,0 (3,808±0,054%). Rata-rata kadar nitrogen residu destilat pada pH awal media pH 4,0 (2,915± 0,033%) tidak berbeda signifikan (P>0,05) dibandingkan dengan rata-rata kadar nitrogen residu destilat pada pH 4,5 (3,410±0,424%), namun demikian signifikan (P<0,05) lebih rendah dibandingkan dengan rata-rata kadar nitrogen residu destilat pada pH awal media pH 5,0 (4,143±0,121%), pH 5,5 (4,445±0,122%), pH 6,0 (4,418±0,132%), pH 6,5

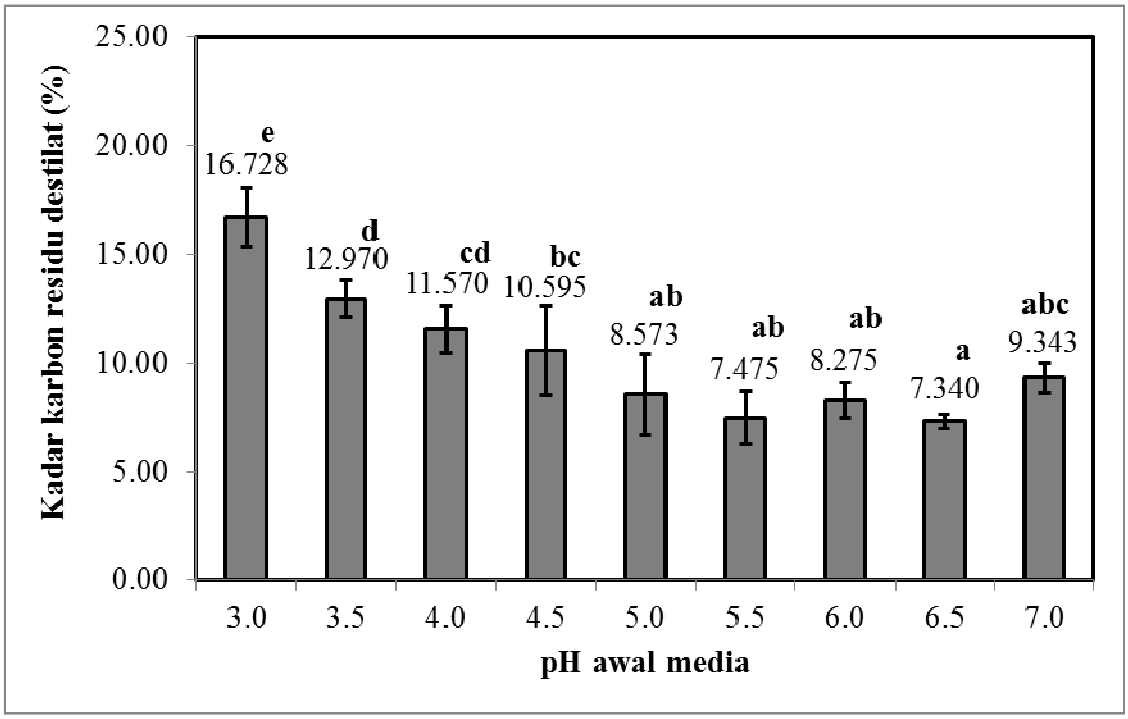
(4,388±0,258%), dan pH 7,0 (3,808±0,054%). Rata-rata kadar nitrogen residu destilat pada pH awal media 5,0 (4,143±0,121%) tidak berbeda signifikan (P>0,05) dibandingkan dengan rata-rata kadar nitrogen residu destilat pada pH awal media pH 5,5 (4,445±0,122%), pH 6,0 (4,418±0,132%), 6,5 (4,388±0,258%) dan pH 7,0 (3,808±0,054%). Rata-rata kadar nitrogen residu destilat pada pH awal media 5,5 (4,445±0,122%), pH 6,0 (4,418±0,132%), dan pH 6,5 (4,388±0,258%) signifikan (P<0,05) lebih tinggi dibandingkan kadar nitrogen residu destilat pada pH 7,0 (3,808±0,054%).

d. Kadar karbon residu destilat

Hasil penelitian kadar karbon residu destilat media hidrolisat sekam padi yang difermentasi dengan ko-kultur *S. cerevisiae* dan *C. tropicalis* pada pH awal yang berbeda disajikan pada Gambar 5.1.6. Kadar karbon residu destilat media hidrolisat sekam padi hasil fementasi ko-kultur *S. cerevisiae* dan *C. tropicalis* signifikan (P<0,05) dipengaruhi oleh pH awal media.

Hasil penelitian memperlihatkan bahwa rata-rata kadar karbon residu destilat pada pH awal media 3,0 (16,728± 1,375%) signifikan (P<0,05) lebih tinggi dibandingkan dengan rata-rata kadar karbon residu destilat pada pH 3,5 (12,970± 0,841%), pH 4,0 (11,570± 1,111%), pH 4,5 (10,595± 2,067%), pH 5,0 (8,573± 1,855%), pH 5,5 (7,475± 1,199%), pH 6,0 (8,275± 0,837%), pH 6,5 (7,340± 0,327%), dan pH 7,0 (9,3438± 0,685%). Rata-rata kadar karbon residu destilat pada pH awal media 3,5 (12,970± 0,841%) tidak berbeda signifikan (P>0,05) dibandingkan dengan rata-rata kadar karbon residu destilat pada pH awal 4,0 (11,570± 1,111%), namun signifikan (P<0,05) lebih tinggi dibandingkan dengan rata-rata kadar karbon residu destilat pada pH awal 4,5 (10,595± 2,067%), pH 5,0 (8,573± 1,855%), pH 5,5 (7,475± 1,199%), pH 6,0 (8,275± 0,837%), pH 6,5 (7,340± 0,327%), dan pH 7,0 (9,3438± 0,685%). Rata-rata kadar karbon residu destilat pada pH awal media 4,0 (11,570± 1,111%) tidak berbeda signifikan (P>0,05) dibandingkan dengan rata-rata kadar karbon residu destilat pada pH pH awal 4,5 (10,595± 2,067%) dan pH 7,0 (9,3438± 0,685%), namun signifikan (P<0,05) lebih tinggi dibandingkan dengan rata-rata kadar karbon residu destilat pada pH 5,0 (8,573± 1,855%), pH 5,5 (7,475± 1,199%), pH 6,0 (8,275± 0,837%) dan pH 6,5

(7,340± 0,327%). Hasil penelitian juga menunjukkan bahwa tidak terdapat perbedaan signifikan (P>0,05) antar rata-rata kadar karbon residu destilat pada pH awal media 5,0 (8,573± 1,855%), pH 5,5 (7,475± 1,199%), pH 6,0 (8,275± 0,837%) dan pH 6,5 (7,340± 0,327%).



Gambar 5.1.5. Kadar karbon residu kering destilat media hidrolisat sekam padi yang difermentasi dengan ko-kultur *S. cerevisiae* dan *C. tropicalis* pada pH awal yang berbeda

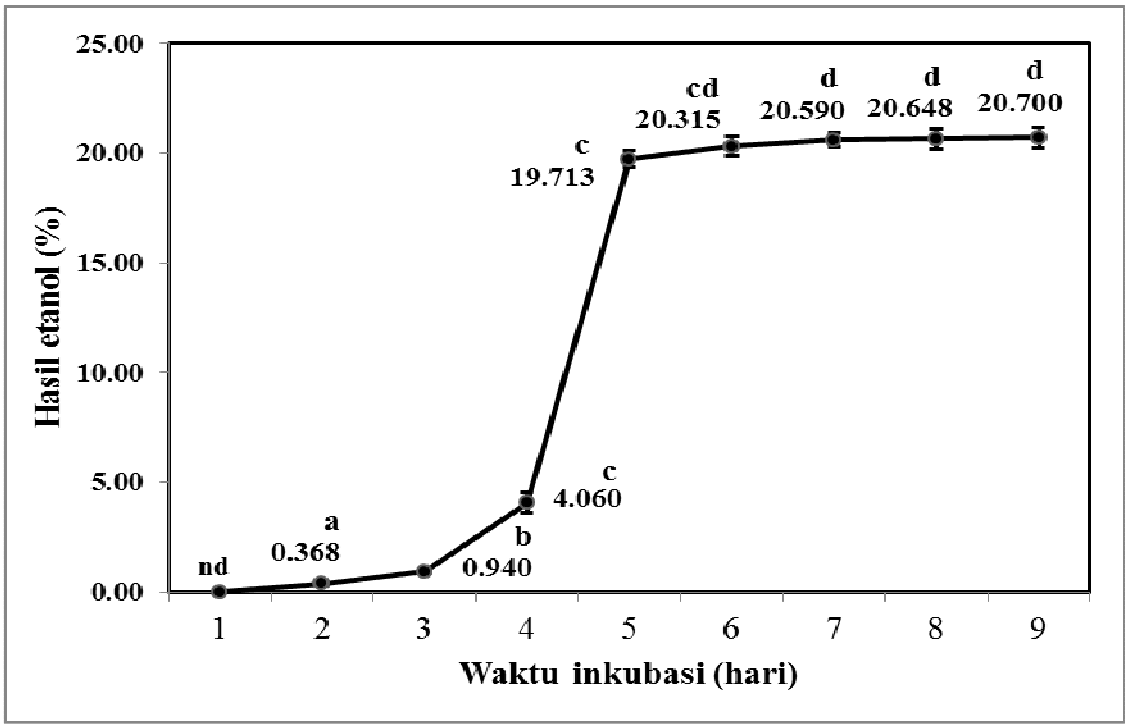
5.1.5 **Efek waktu inkubasi**

a. Hasil etanol

Hasil penelitian menunjukkan bahwa waktu inkubsi berpengaruh signifikan (P<0,05) terhadap etanol hasil fermentasi ko-kultur *S. cerevisiae* dan *C. tropicalis* dari media hidrolisat sekam padi.

Gambar 5.1.6 memperlihatkan bahwa pada waktu inkubasi 1 hari tidak terdeteksi hasil etanol. Hasil etenol pada waktu inkubasi 2 hari (0,368 ± 0,11%) signifikan (P<0,05) lebih rendah dibandingkan dengan hasil etanol pada waktu inkubasi 3 hari (0,940± 0,053%), 4 hari (4,060±0,456%), 5 hari (19,713± 0,383%), 6 hari (20,315± 0,416%), 7 hari (20,590± 0,329%), 8 hari (20,648± 0,482%), dan 9 hari (20,700±

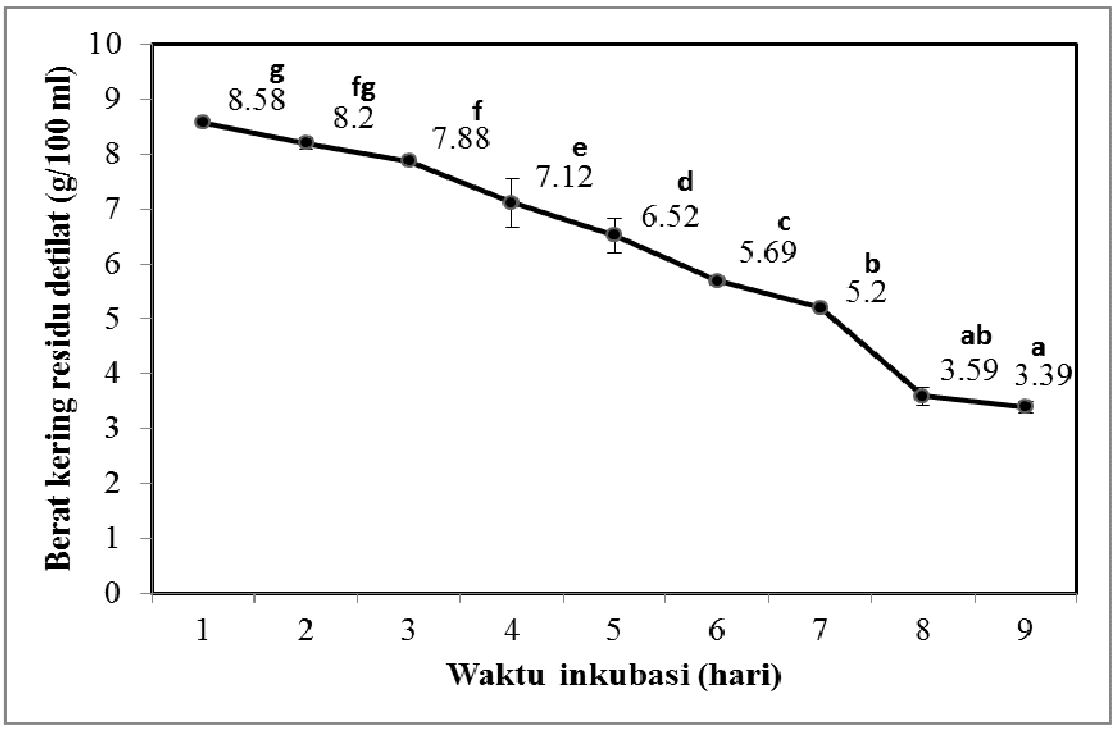
0,463%). Hasil etenol pada waktu inkubasi 3 hari (0,940± 0,053%) signifikan (P<0,05) lebih rendah dibandingkan dengan hasil etanol pada waktu inkubasi 4 hari (4,060±0,456%), 5 hari (19,713± 0,383%), 6 hari (20,315± 0,416%), 7 hari (20,590± 0,329%), 8 hari (20,648± 0,482%), dan 9 hari (20,700± 0,463%). Hasil etenol pada waktu inkubasi 4 hari (4,060±0,456%) signifikan (P<0,05) lebih rendah dibandingkan dengan hasil etanol pada waktu inkubasi 5 hari (19,713± 0,383%), 6 hari (20,315± 0,416%), 7 hari (20,590± 0,329%), 8 hari (20,648± 0,482%), dan 9 hari (20,700± 0,463%). Hasil etenol pada waktu inkubasi 5 hari (19,713± 0,383%) tidak berbeda signifikan (P>0,05) dibandingkan dengan hasil etanol pada waktu inkubasi, 6 hari (20,315± 0,416%), namun signifkan (P<0,05) lebih rendah dibandingkan dengan hasil etanol pada waktu inkubasi 7 hari (20,590± 0,329%), 8 hari (20,648± 0,482%), dan 9 hari (20,700± 0,463%). Penelitian ini menunjukkan tidak terdapat perbedaan yang signifikan (P>0,05) antara hasil etenol pada waktu inkubasi 6 hari (20,315± 0,416%), 7 hari (20,590± 0,329%), 8 hari (20,648± 0,482%), dan 9 hari (20,700± 0,463%).



Gambar 5.1.6. Hasil etanol dari media hidrolisat sekam padi yang difermentasi dengan ko-kultur *S. cerevisiae* dan *C. tropicalis* pada waktu inkubasi yang berbeda

b. Berat kering residu destilat

Hasil penelitian menunjukkan bahwa waktu inkubsi berpengaruh signifikan (P<0,05) terhadap berat kering residu destilat media hidrolisat sekam padi yang difermentasi dengan ko-kultur *S. cerevisiae* dan *C. tropicalis.* Hasil penelitian (Gambar 5.1.7) memperlihatkan berat kering residu destilat menurun seiring dengan penambahan waktu inkubasi.



Gambar 5.1.7. Berat residu kering destilat media hidrolisat sekam padi yang difermentasi dengan ko-kultur *S. cerevisiae* dan *C. tropicalis* pada waktu inkubasi yang berbeda

Berat kering residu destilat pada waktu inkubasi 9 hari (3,39±0.11 g/100 ml) signifikan (P<0,05) lebih rendah dibandingkan dengan berat kering residu destilat pada waktu inkubasi 7 hari (5,20±0.04 g/100 ml), 6 hari (5,69±0.32 g/100 ml), 5 hari (6,52±0.32 g/100 ml), 4 hari (7,12±0.44 g/100 ml), 3 hari (7,88±0.04 g/100 ml), 2 hari (8,20±0.10 g/100 ml) dan 1 hari (8,58±0.04 g/100 ml), namun tidak berbeda signifikan (P>0,05) dengan berat kering residu destilat pada waktu inkubasi 8 hari (3,60±0.17 g/100 ml). Berat kering residu destilat pada waktu inkubasi 1 hari (8,58±0.04 g/100 ml) signifikan (P<0,05) lebih tinggi dibandingkan dengan berat kering residu destilat pada waktu 3, 4, 5, 6, 7, 8 dan 9 hari, namun tidak berbeda signifikan (P>0,05) dibandingkan berat kering

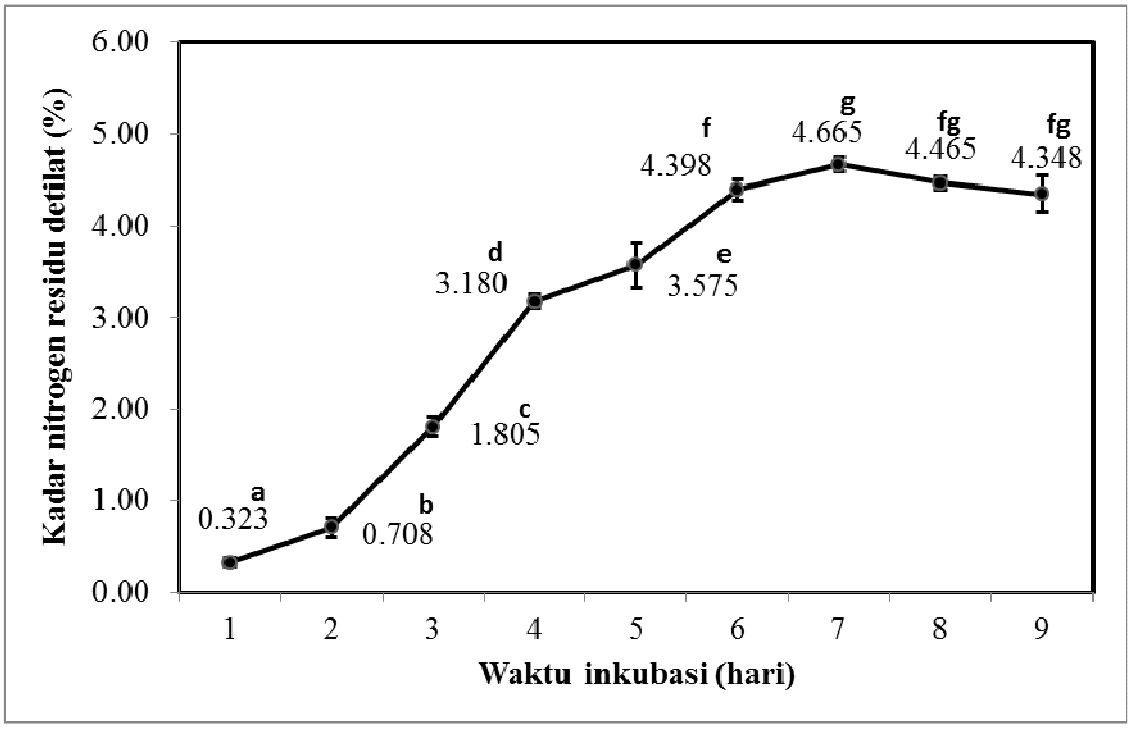
residu destilat pada waktu inkubasi 2 hari dan berat kering residu destilat 2 hari tidak berbeda signifikan (P>0,05) dengan berat kering residu destilat 3 hari.

c. Kadar nitrogen residu destilat

Hasil penelitian menunjukkan bahwa waktu inkubsi berpengaruh signifikan (P<0,05) terhadap kadar nitrogen residu destilat media hidrolisat sekam padi yang difermentasi dengan ko-kultur *S. cerevisiae* dan *C. tropicalis.* Hasil penelitian (Gambar 5.1.8) memperlihatkan kadar nitrogen residu destilat meningkat seiring dengan penambahan waktu inkubasi sampai 7 hari, selanjutnya kadar nitrogen residu destilat relatif konstan sampai waktu inkubasi 9 hari.

Kadar nitrogen residu destilat pada waktu inkubasi 1 hari (0,323± 0,046%) signifikan (P<0,05) lebih rendah dibandingkan dengan kadar nitrogen residu destilat pada waktu inkubasi 2 hari (0,708± 0,105%), 3 hari (1,805± 0.104%), 4 hari (3,180± 0,075%), 5 hari (3,575± 0,247%), 6 hari (4,398± 0,110%), 7 hari (4,665± 0,080%), 8 hari (4,4655±0,065%) dan 9 hari (4,348±0,198%). Kadar nitrogen residu destilat pada waktu inkubasi 2 hari (0,708± 0,105%) signifikan (P<0,05) lebih rendah dibandingkan dengan kadar nitrogen residu destilat pada waktu inkubasi 3 hari (1,805± 0.104%), 4 hari (3,180± 0,075%), 5 hari (3,575± 0,247%), 6 hari (4,398± 0,110%), 7 hari (4,665± 0,080%), 8 hari (4,4655±0,065%) dan 9 hari (4,348±0,198%). Kadar nitrogen residu destilat pada waktu inkubasi 3 hari (1,805± 0.104%) signifikan (P<0,05) lebih rendah dibandingkan dengan kadar nitrogen residu destilat pada waktu inkubasi 4 hari (3,180± 0,075%), 5 hari (3,575± 0,247%), 6 hari (4,398± 0,110%), 7 hari (4,665± 0,080%), 8 hari (4,4655 ± 0,065%) dan 9 hari (4,348 ± 0,198%). Kadar nitrogen residu destilat pada waktu inkubasi 4 hari (3,180± 0,075%) signifikan (P<0,05) lebih rendah dibandingkan dengan kadar nitrogen residu destilat pada waktu inkubasi 5 hari (3,575± 0,247%), 6 hari (4,398± 0,110%), 7 hari (4,665± 0,080%), 8 hari (4,4655±0,065%) dan 9 hari (4,348±0,198%). Kadar nitrogen residu destilat pada waktu inkubasi 5 hari (3,575± 0,247%) signifikan (P<0,05) lebih rendah dibandingkan dengan kadar nitrogen residu destilat pada waktu inkubasi 6 hari (4,398± 0,110%), 7 hari (4,665± 0,080%), 8 hari (4,4655±0,065%) dan 9 hari (4,348±0,198%). Kadar nitrogen residu destilat pada waktu inkubasi 6 hari (4,398± 0,110%) signifikan (P<0,05) lebih rendah dibandingkan dengan

kadar nitrogen residu destilat pada waktu inkubasi 7 hari (4,665± 0,080%), namun tidak berbeda signifikan (P>0,05) dibandingkan dengan kadar nitrogen pada waktu inkubasi 8 hari (4,4655±0,065%) dan 9 hari (4,348±0,198%). Tidak terdapat perbedaan yang signifikan (P>0,05) antar kadar nitrogen residu destilat pada waktu inkubasi 7 hari (4,665± 0,080%) 8 hari (4,4655±0,065%) dan 9 hari (4,348±0,198%).



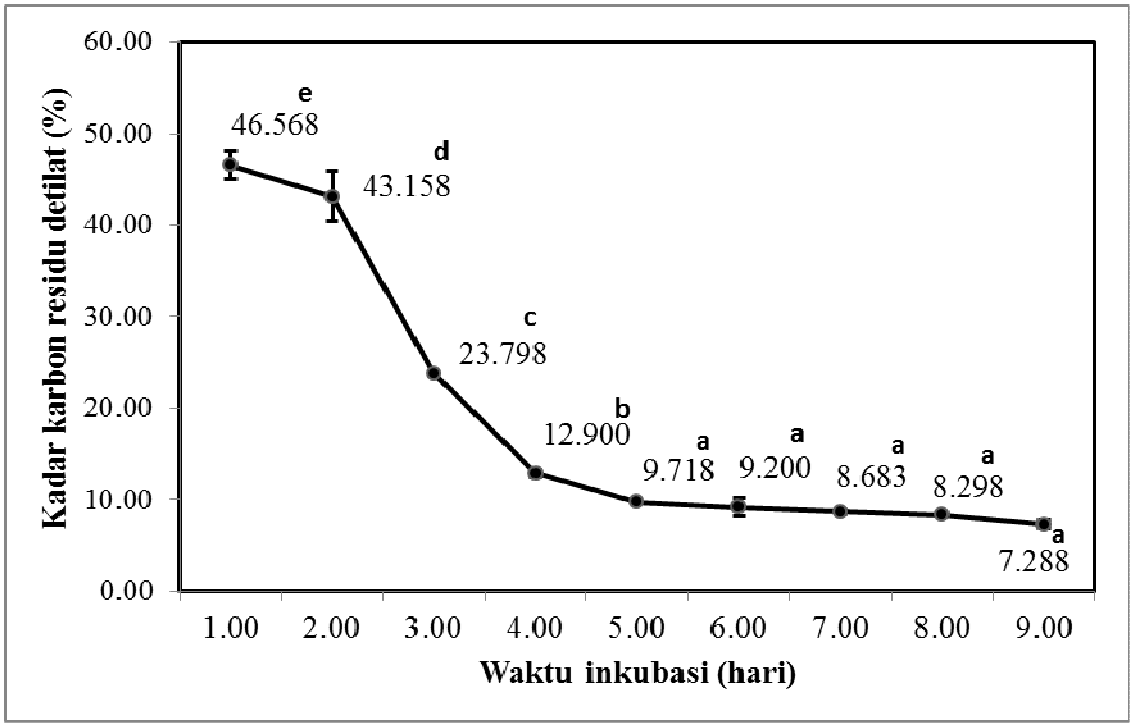
Gambar 5.1.7. Kadar nitrogen residu kering destilat media hidrolisat sekam padi yang difermentasi dengan ko-kultur *S. cerevisiae* dan *C. tropicalis* pada waktu inkubasi yang berbeda

d. Kadar karbon residu destilat

Hasil penelitian menunjukkan bahwa waktu inkubasi berpengaruh signifikan (P<0,05) terhadap kadar karbon residu destilat media hidrolisat sekam padi yang difermentasi dengan ko-kultur *S. cerevisiae* dan *C. tropicalis.* Hasil penelitian (Gambar 5.1.9) memperlihatkan kadar karbon residu destilat meurun seiring dengan penambahan waktu inkubasi.

Kadar karbon residu destilat pada waktu inkubasi 1 hari (46,568±1,567%) signifikan (P<0,05) lebih tinggi dibandingkan dengan kadar karbon residu destilat pada waktu inkubasi 2 hari (43,158± 2,676%), 3 hari (23,798± 0,262%), 4 hari (12,900±

0,414%), 5 hari (9,718± 0,050%), 6 hari (9,200± 1,001%), 7 hari (8,683± 0,250%), 8 hari (8,298± 0,072%) dan 9 hari (7,288± 0,470%). Kadar karbon residu destilat pada waktu inkubasi 2 hari (43,158± 2,676%) signifikan (P<0.05) lebih tinggi dibandingkan dengan kadar nitrogen pada waktu inkubasi 3 hari (23,798± 0,262%), 4 hari (12,900± 0,414%), 5 hari (9,718± 0,050%), 6 hari (9,200± 1,001%), 7 hari (8,683± 0,250%), 8 hari (8,298± 0,072%) dan 9 hari (7,288± 0,470%). Kadar karbon residu destilat pada waktu inkubasi 3 hari (23,798± 0,262%) signifikan (P<0,05) lebih tinggi dibandingkan dengan kadar nitrogen pada waktu inkubasi 4 hari (12,900± 0,414%), 5 hari (9,718± 0,050%), 6 hari (9,200± 1,001%), 7 hari (8,683± 0,250%), 8 hari (8,298± 0,072%) dan 9 hari (7,288± 0,470%). Kadar karbon residu destilat pada waktu inkubasi 4 hari (12,900± 0,414%) signifikan (P<0,05) lebih tinggi dibandingkan dengan kadar karbon residu destilat pada waktu inkubasi 5 hari (9,718± 0,050%), 6 hari (9,200± 1,001%), 7 hari (8,683± 0,250%), 8 hari (8,298± 0,072%) dan 9 hari (7,288± 0,470%). Kadar karbon residu destilat pada waktu inkubasi 5, 6, 7, 8 dan 9 hari tidak menunjukkan perbedaan yang signifikan (P>0,05) walaupun secara bertahap menurun.



Gambar 5.1.8. Kadar karbon residu kering destilat media hidrolisat sekam padi yang difermentasi dengan ko-kultur *S. cerevisiae* dan *C. tropicalis* pada waktu inkubasi yang berbeda

5.2. Luaran yang telah dicapai

Luaran hasil penelitian tahun ketiga belum dapat dicapai karena penelitian tahun ketiga belum selesai. Namun demikian, pada penelitian tahun kesatu dan kedua telah dicapai adalah:

1. Publikasi artikel ilmiah dalam Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 17, No. (3) : 2015 : 577-586 dengan judul "SUGAR CONSUMPTION IN MONO AND CO-CULTURE SACCHAROMYCES CEREVISIAE AND OTHERS SELECTED MICROORGANISM FOR BIOETHANOL PRODUCTION FROMSTREAM RICE HUSK MEDIUM"
2. Publikasi ilmiah dalam the Journal of Microbiology, Biotechnology and Food Sciences dengan judul "ETHANOL PRODUCTION AND SUGARS CONSUMTION OF CO-CULTURE *Saccharomyces cerevisiae* FNCC 3012 WITH *Candida tropicalis* FNCC 3033 IN MEDIA CONTAINING INHIBITORS FERMENTATION (under review)
3. Publikasi ilmiah dalam the African Journal Microbiology Research dengan judul "IMPROVED ETHANOL PRODUCTION BY CO-CULTURE FERMENTATION *Saccharomyces cerevisiae* WITH *Candida tropicalis* FROM RICE HUSK HYDROLYSATE MEDIA USING SUPLEMENTATION VARIOUS INORGANIC NITROGEN SOURCES AND MOLASSES (under review)
4. Bagian materi buku ajar "Mikrobiologi Pangan" dengan Penerbit Andi Ofset
5. Bagian Materi buku ajar "Mikologi" (Draff)
6. Makalah seminar nasional :Proceeding Trend Implimentasi Halal di Indonesia dengan judul "Potensi ko-kultur Sacharomyces cerevisiae dan Candida tropicalis untuk produksi bioethanol dari materi lignoselulosa"

BAB 6. RENCANA TAHAPAN BERIKUTNYA

Tahapan selanjutnya akan direncanakan untuk melanjutkan publikasi artikel pada jurnal internasional (revisi) dan menulis buku ajar Mikologi.

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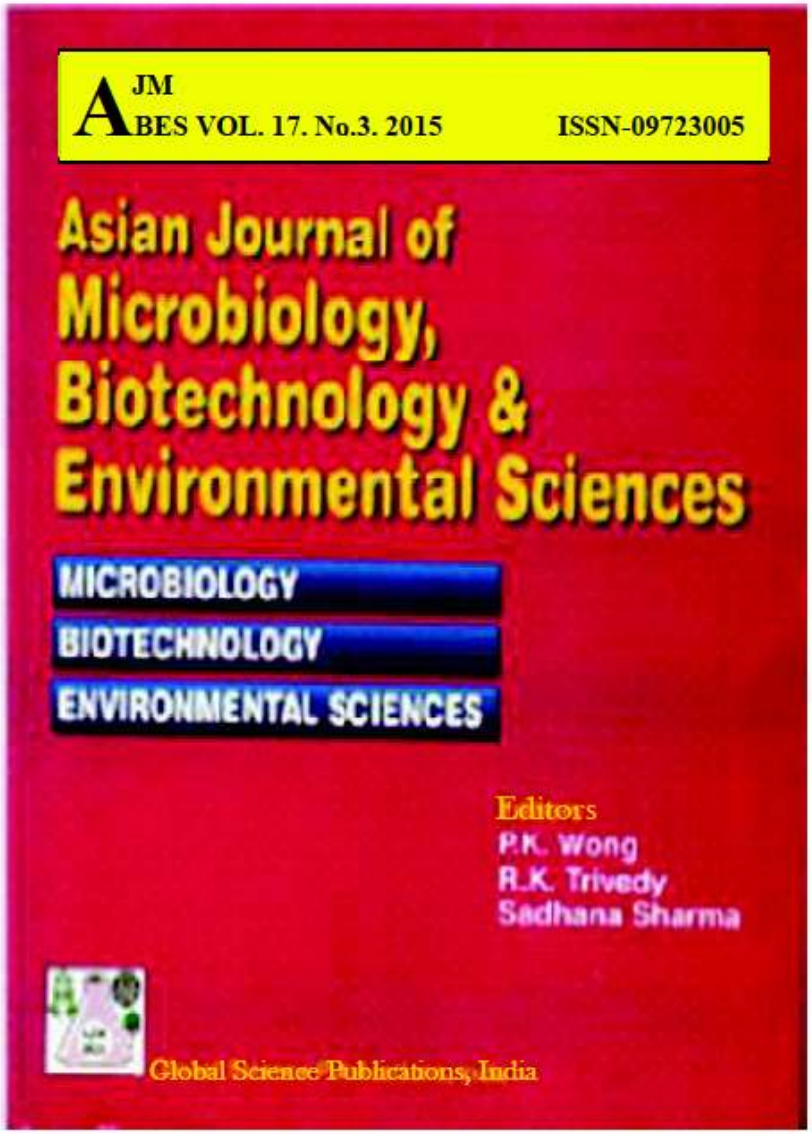

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SUGAR CONSUMPTION IN MONO AND CO-CULTURE *SACCHAROMYCES CEREVISIAE* AND OTHERS SELECTED MICROORGANISM FOR BIOETHANOL PRODUCTION FROM STREAM RICE HUSK MEDIUM

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Key words : Co-culture, Rice husk, Ethanol, S. cerevisiae, C. tropicalis, Z. Mobilis and P. resticulosum

Abstract–This study sought to explore potential of mono and co-culture fermentation of *S.cerevisiae* with *C. tropicalis, Z. mobilis* and *P. resticulosum* for bioethanol production from stream rice husk medium. Batch fermentation was conducted in a waste rice husk hydrolysate basal medium with 4 g/L urea, 3 g/L $NaNO_3$, 3 g/L NH_4NO_3, 1 g/L KH_2PO_4 and 0.7 g/L $MgSO_4.7H_2O$ supplemented and 3 days (30°C, 60-70% relative humidity, dark, 150 rpm agitation) incubation. Maximum ethanol production (2.125±0,259 %/L) was gained from co-culture *S.cerevisiae, C. tropicalis* with efficiency fermentation 89.25±10.95%. Co-culture *S.cerevisiae, C. tropicalis* have ability to generate and convert fermentable sugars from a waste stream rice husk to ethanol. Co-culture *S. cerevisiae, C. tropicalis* demonstrated high potential and such application provides a process for agricultural waste stream resource reuse for production of ethanol in increasing demand.

INTRODUCTION

Paddy is primary Indonesia crops with annual production of approximately 68,956,292 metric tons (BPS, 2012). Rice husks are a lignocellulosic waste product representing 20-22% of paddy by weight. Quantitively, rice husk have high potential as feedstock for industrial production include bioethanol. Utilization of lignocellulosic materials as feedstock for bioethanol production is an alternative strategic to reduce production cost and solve technological problems. Conversion of lignocellulosic material including agricultural waste to bioethanol is important choice to exploitation of alternative energy resources and reduces air pollution (Sa´nchez and Cardona, 2008; Patel *et al.*, 2012).

Indonesia annual bioethanol production of approximately 200.000 kl using sugars cane, cassava, sweet potato and corn (Panaka and Yudiarto, 2007). However, ethanol production from starch or sugar has potentially competes with food production either directly or indirectly by competing with food production for land and water (Chen, 2011). The ability to generate and convert fermentable sugars from lignocellulosic materials to ethanol is the central technological challenge (Wan *et al.*, 2012; Chandra *et al.*, 2007; Dien *et al.*, 2003). Strategies which require a single organism to convert xylose and glucose simultaneously suffer from several limitations. One limitation is that despite the presence of the genetic apparatus to consume both sugars, glucose remains the preferred substrate, and the consumption of the sugars is asynchronous (Eiteman *et al.*, 2008). Currently, co-culture fermentation is development strategic to increasing cellulose hydrolysis rate, enrichment substrate utilization and increasing ethanol production via different metabolic pathway combination to reduce negative effect of inhibitor (Cheng and Zhu, 2012). In this study, we examined sugar uptake and ethanol production from a stream waste rice husk by mono

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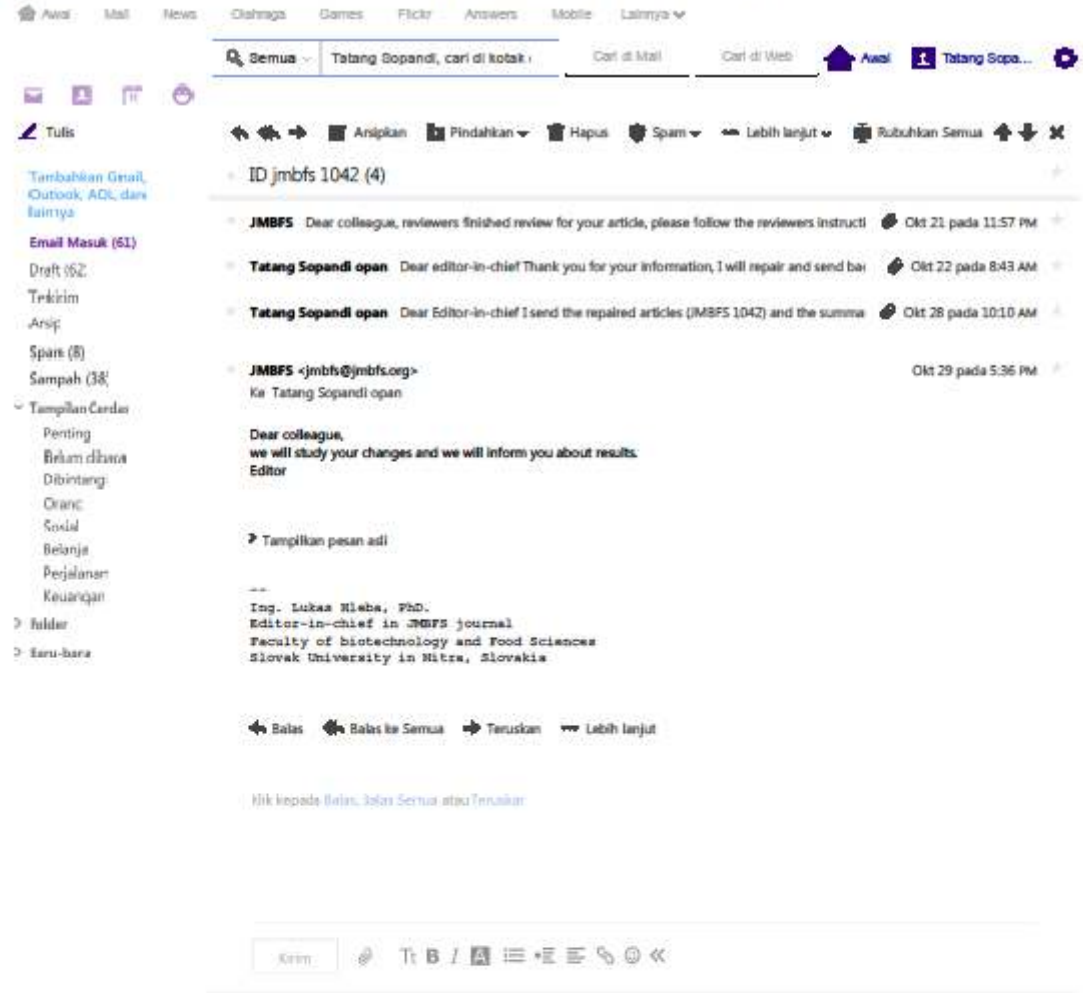
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ETHANOL PRODUCTION AND SUGARS CONSUMTION OF CO-CULTURE *Saccharomyces cerevisiae* FNCC 3012 WITH *Candida tropicalis* FNCC 3033 IN MEDIA CONTAINING INHIBITORS FERMENTATION

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Inhibitor fermentation is one of the problems that arise in the ethanol production from lignocellulose waste. This work examined ethanol yield and sugar consumption of mono and co-culture *S. cerevisiae* with *C. tropicalis* in media containing inhibitors fermentation. Furfural and phenol were used inhibitor fermentation in basal medium with concentration 2.0 and 5.0 %, respectively. The basal medium contained 20 $g.L^{-1}$ glucose, 20 $g.L^{-1}$ D (+) xylose, 20 $g.L^{-1}$ arabinose, 4 $g.L^{-1}$ urea, 3 $g.L^{-1}$ $NaNO_3$, 3 $g.L^{-1}$ NH_4NO_3, 1 $g.L^{-1}$ KH_3PO_4 and 0.7 $g.L^{-1}$ $MgSO_4 \cdot 7H_2O$ and pH adjusted 5.5 with 1 $mol.L^{-1}$ HCl. After furfural or phenol addition separately, and inoculated by mono and co-culture *S. cerevisiae* FNCC 3012 with *C. tropicalis* FNCC 3033, all media incubated at 28–29°C, 50% r.h. in the dark for 5 days in a rotary incubator at 60 rev min. We found yeast colony count, sugar consumption, ethanol yialds and efficiency of fermentation by co-culture *S. cerevisiae* with *C. tropicalis* higher than mono-culture *S. cerevisiae* or *C.tropicalis* in the fermentation media with or without inhibitors. This work indicated that co-culture *S. cerevisiae* with *C. tropicalis* more tolerance to furfural and phenol. In basal medium, basal medium plus 2.5 or 5.0% furfural, ethanol yield 8.52%, 5.37% and 3.83%, respectively and efficiency fermentation 27.00%, 17.00% and 12.20%. In basal medium, basal medium plus 2.5 or 5.0% phenol, ethanol yields 8.13%, 5.62% and 3.19%, respectvely and efficiency fermentation 28.20%, 20.00% and 14.00%. Co-culture *S. cerevisiae* FNCC 3012 with *C. tropicalis* 3033 demonstrated potential as a fermentation process for ethanol production from lignocelulosic medium or media content inhibitors. The use of this co-culture effectively utilize hexose and pentose sugars in the substrate, increasing the yield and efficiency of fermentation for ethanol production.

Key words : co-culture, fermentation, ethanol, inhibitor, *S.cerevisiae*, and *C.tropicalis*

INTRODUCTION

The fossil fuels limitation and environmental condition have attention developing alternative energy sources that lower impact on the environment (**Abreu-Cavalheiro and Monteiro, 2013**). Ethanol is an alternative energy source has the potential to replace fossil energy sources and received high attention over the past few years (**Chen, 2011**). Ethanol can be produced from various agricultural raw materials including lignocellulose (**Balat, 2011; Tesfaw and Assefa, 2014**). Due to renewability, large quantities, relatively low prices than grain or sugar, and potential environmental benefits, lignocellulosic biomass has been considered as possible raw material for ethanol production (**Cardona and Sa´nchez, 2007; Kumar *et al*., 2008; Lee and Huang, 2000; Mielenz, 2001; Service, 2007; Zaldivar *et al*,. 2001**). Preferably lignocellulosic biomass as raw material for ethanol production compared with sugar or starch derived from crops products because it does not compete as food needs and concern the utilization of agricultural residue (**Gutiérrez-Rivera *et al*,. 2012; Ishola *et al*,. 2014**).

Efficient fermentation of ethanol production from lignocellulosic biomass is affected by the consumption of glucose and xylose which is the main product of lignocellulosic hydrolysates (**Lee and Huang, 2000; Service, 2007, Eiteman *et al.*, 2008**). However, the lack of a microorganism able to ferment efficiently all sugars released by hydrolysis from lignocellulosic materials has been one of the main factors preventing utilization of lignocellulose (**Zaldivar *et al*., 2001**). *Saccharomyces cerevisiae* is the dominant yeast used for ethanol production can not metabolize xylose and convert it into ethanol (**Jeffries and Jin, 2004; Lin and Tanakan, 2006**).

In addition, others problem associated with efficient conversion of cellulose and hemicellulose sugars to ethanol is that during dilute acid hydrolysis a broad range of compounds which inhibit the fermenting microorganism are liberated or formed along with the sugars (**Larsson *et al*., 2001**). The presence of inhibiting compounds, such as weak acids, furans and phenolic compounds that formed or released during thermo-chemical pre-treatment step such as acid and steam explosion can decreased ethanol yield and productivity of fermentation lignocellulosic (**Parawira and Tekere, 2011**). Reducing in the ethanol yield and productivity by

inhibiting components can be caused influence on the performance of microorganisms during fermentation step (**Almeida *et al*., 2007**).

The choice of the fermenting microorganism, complete substrate utilization, inhibitor tolerance and ethanol productivity are important aspects in ethanol production from lignocellulose (**Bettiga *et al*., 2009**). Microorganisms that consume sugars such as glucose and xylose sequentially must have lower productivities for the generation of a product than if the organism were to consume the sugars simultaneously (**Zaldivar *et al*., 2001**). For economical bioethanol production from lignocellulosic materials, The microorganism should use efficiently all glucose and xylose in the lignocellulose hydrolysate and the microorganism should have high tolerance to the inhibitors present in the lignocellulose hydrolysate (**Cheng *et al*., 2014**).

Strategies of using a single microorganism to convert glucose and xylose simultaneously have limitations (**Eiteman *et al*., 2008**). The co-culture among microorganisms could potentially increase ethanol production and efficiency of fermentation from hydrolyzate lignocellulosic hydrolysate. Co-culture of *S. cerevisiae* and other microorganism reduced inhibitory compounds in lignocellulosic hydrolysates (**Taherzadeh *et al*., 2013; Wan *et al*., 2012**), increased ethanol yield and production rate (**Singh et al., 2014; Wan 2012**), shortens fermentation time, and reduced process cost (**Hickert et al., 2013; Tesfaw and Assefa, 2014**). Co-culture *S.cerevisiae* with *C. tropicalis* have ability to generate and convert fermentable sugars from a waste stream rice husk to ethanol (**Sopandi and Wardah, 2015**). This work examined ethanol yield and sugar consumption of mono and co-culture *S. cerevisiae* with *C. tropicalis* in medium containing inhibitors fermentation.

MATERIAL AND METHODS

Culture microorganism

Saccharomyces cerevisiae FNCC 3012 and *Candida tropicalis* FNCC 3033 were obtained from Microbiology Laboratories, PPAU Gadjah Mada University, Yogyakarta, Indonesia. Saubroad agar (Oxoid) was used to maintain the strain *S. cerevisiae* and *C. tropicalis*. Working stock cultures were prepared from stock, 7 days, 28ºC SA plate cultures subcultures from the master stock. Colonies were aseptically sampled by scraping the top with an inoculating loop and transfer to 10 ml sterile water. Inoculums stock suspension were prepared from working stock and diluted to 1.7 x 10^6 cell.mL^{-1}, as enumerated using a haemocytometer.

Fermentation

The batch fermentation experiments were carried out in 250 mL Erlenmeyer flask with working volumes of 100 mL. The basal medium contained 20.0 g.L^{-1} glucose, 20.0 g.L^{-1} D (+) xylose, 20.0 g.L^{-1} arabinose, 4.0 g.L^{-1} urea, 3.0 g.L^{-1} $NaNO_3$, 3.0 g.L^{-1} NH_4NO_3, 1.0 g.L^{-1} KH_3PO_4 and 0.7 g.L^{-1} $MgSO_4 \cdot 7H_2O$ and pH adjusted 5.5 with 1.0 mol.L^{-1} HCl. Liquid basal medium (9.0 l) were mixed thoroughly and 100 ml individually dispensed into 250-mL Erlenmeyer flasks, autoclaved, cooled to room temperature. Media in Erlenmeyer was divided into 2 groups, furfural was added to individual flasks and final concentrations of 0.0, 2.5 and 5.0% into ones group and another group, phenol was added to individual flasks and final concentration of 0.0, 2.5, and 5.0%. Aseptically dispensed into individual Erlenmeyer flasks, 1.0 mL inoculum stock suspension of *S. cerevisiae* and 1.0 mL *C. tropicalis* for mono culture, respectively and 0.5 mL *S. cerevisiae* with 0.5 mL *C. tropicalis* for co-culture added into the flasks and incubated at 28–29°C, 50% r.h. in the dark for 5 d in a rotary incubator at 60 rev min. This inoculation and incubation method was used for all cultivation in this study.

Yeast count

To observed yeast count, 10 mL media before and after fermentation (5 d) was pipetted and serial dilution 10^{10} in sterile water. Each serial dilution (0.1 mL) inoculated and spread onto Saubroad agar (Oxoid), incubated at 28–29°C, 50% r.h. in the dark for 3 d. Colony counter was used to counting colony on media.

Determination of ethanol

Ethanol were measured using a gas chromatograph Carbomax t70-10-0 column, FID t220 detector, helium as carrier gas with flow rate 40.3 mL.min^{-1}, tin column Porapack Q, detector temperature at 160ºC and column temperature at 180ºC with injection volume 1.0 µL. Fermented media were filtered through Whatmann No.1 paper.

Determination of sugar

Glucose, D(+) xylose and arabinose were determined using HPLC (Shimadzu, Kyoto, Japan) at 85ºC, a Metacharb 87C column, H_2O as eluent, flow rate 0.6 mL/min and RID detector. After fermentation, medium was mixture and aseptically filtered through

Whatmann No.1 paper. Filtrate were centrifuged at 12,000 rpm for 15 min, refiltered through millex 0.45 µm and 25 µL of sample injected to HPLC. Standard was used glucose, D(+) xylose and arabinose (Merck) with concentration 62.5, 125, 250 and 500 ppm, respectively, as a standard.

Efficiency fermentation

To determinate of efficiency fermentation ethanol production by mono and co-culture *S. cerevisiae* and *C. tropicalis*, we used formula:

$$EF_{biomass}(\%) = \frac{\text{Ethanol yields (L)}}{\text{Amount sugar before fermentation (g) x 0,511}} \text{ x } 100$$

Statistical analysis

Tukey's honestly significant difference multiple comparison test and paired sample t-test were used to segregate significantly different treatment using SPSS 16 software. Analysis variance (ANOVA) was performed to determine significant differences between experiments ($P<0.05$).

RESULTS AND DISCUSSION

Yeast count

Addition of furfural (Fig 1A) to the growth medium significantly ($p<0.05$) decreased yeast colony count in mono or co-culture *S. cerevisiae* with *C. tropicalis*. This work indicated furfural inhibits growth of yeast in mono-culture and co-culture *S. cerevisea* with *C. tropicalis*. Some investigators have reported the effect of furfural on the growth of microorganisms. **Palmqvist and Hahn-Ha¨gerdal (2000)** reported furfural inhibited on the specific growth and fermentation rate of yeasts. **Agbogbo *et al.* (2007)** reported concentration of furfural 1.5 $g.L^{-1}$ could interfere respiration and growth of microorganisms. **Hristozova *et al.* (2000)** reported concentration furfural 0.04% inhibited glutamate dehydrogenase and ion ammonia assimilation at alanine metabolism of *C.blankii* 35 and *C. pseudotropicalis* 11. **Kelly *et al.* (2008)** reported concentration furfural 1 $g.L^{-1}$ or higher inhibited growth of *C. guilliermondii*. **Jones (1989)** and **Almeida *et al.* (2009)** suggested furfural and 5-hydroxymethyl furfural (HMF) can consume by *S. cerevisiae* with consequence ATP sole. **Mattam *et al.* (2016)** reported increasing of the level of furfural, HMF and acetic acid in growth media led to gradual decrease in *C. tropicalis* biomass.

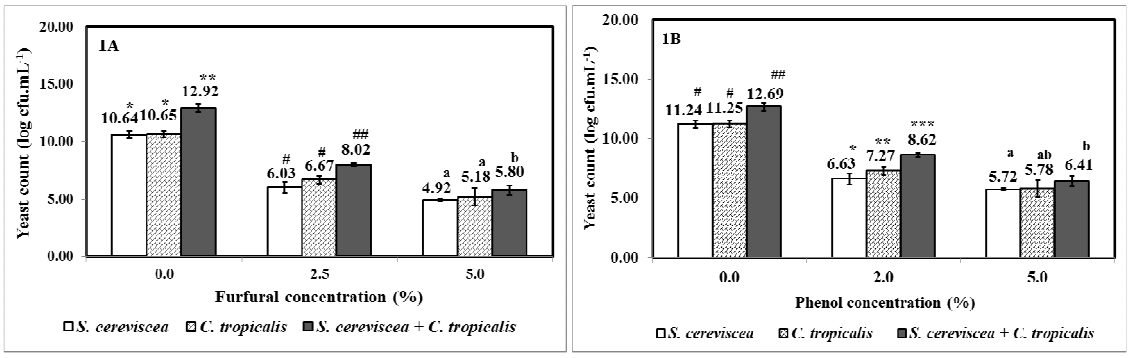

Figure 1 Yeast count in medium plus different concentration of furfural (1A) and phenol (1B) after five days fermentation by mono and co-culture *S. cerevisiae* with *C. tropicalis,* the values are means of 5 independent observations in same furfural concentration different superscripts (^{a} and ^{b}, * and ** or $^{\#}$ and $^{\#\#}$) differ significantly ($p<0.05$).

However, this work show yeast count colony in co-culture *S. cerevisiea* with *C. tropicalis* significantly ($P<0.05$) higher than mono-culture *S. cerevisiae* or *C. tropicalis* in the medium growth with or without furfural. We hypothesized there are synergistic mechanism to stimulate growth yeast through simultaneous utilization of fermentable sugars such as glucose and xylose and degrade furfural in growth medium by co-culture *S. cerevisiae* with *C. tropicalis.* Some investigators have been reported consumption of fermentable sugars (glucose and xylose) simultaneously by co-culture fermentation. **Hikert *et al.* (2013)** reported co-culture *C. shehatae* HM 52.2 with *S. cerevisiae* ICV D254 can simultaneously consumed glucose and xylose in synthetic medium and rice hull hydrolysate. **Fu and Peiris (2008)** reported co-culture *Zymomonas mobilis* with *Pachysolen tannophilus* fully consumed in a mixture of glucose and xylose media. Some investigators have been reported the degradation and conversion of furfural by yeast.

Taherzadeh *et al.* (1999) reported *S. cerevisiae* converted furfural to furfuryl alcohol at exponentially growing cells. **Cheng *et al.* (2014)** reported *C. tropicalis* W103 degraded furfural and 5-hydromethylfurfural under aerobic conditions after 60 h aerobic incubation. Some investigators reported co-culture *S. cerevisiae* with other microbes can reduces inhibitory compounds in lignocellulosic hydrolysates. **Tomás *et al.* (2013)** reported co-culture *Thermoanaerobacter pentosaceus* with *S. cerevisiae* was able to growth and metabolize furfural up to 0.5 $g.L^{-1}$ in the liquid fraction of alkaline-peroxide pretreated rapeseed straw. **Wan *et al.* (2012)** reported co-cultures of *S. cerevisiae* Y5 with *Pichia stipitis* CBS6054 can growth and metabolized furfural and HMF in medium of xylose and glucose mixture.

Addition of phenol (Fig 1B) to the growth medium significantly ($p<0.05$) decreased yeast colony count in mono or co-culture *S. cerevisiae* with *C. tropicalis*. This work indicated phenol inhibits growth of yeast in mono-culture and co-culture *S. cerevisiae* with *C. tropicalis*. Some investigators have reported the inhibitory effect of phenol on the growth of microorganisms. **Heipieper *et al.* (1994**) reported phenol component can degrade cell membrane integrity and decrease membrane affection as buffer selective. **Ding *et al.* (2011)** sugested acetic acid, furfural and phenol are main inhibitor on growth, fermentation and some yeast metabolite. Some inhibitors fermentation such as HMF and phenol component can inhibited yeast metabolism (**Sluiter *et al.*, 2010; Palmqvist and Hahn-Hägerdal, 2000; Almeida *et al.*, 2009; Larsson *et al.*, 1999**). However, **Paca *et al.* (2002)** suggested *C. tropicalis* can use phenol as carbon source and energy. **Adeboye *et al.* (2014)** reported phenolic compounds can exhibit lag phase elongation and decreased maximum specific growth rate of *S. cerevisiae*. **Pizzolitto *et al.* (2015)** reported inhibitory effect of phenol on growth parameter of *Aspergillus parasiticus* depend on the compound assayed and its concentration in the medium. Similar furfural, this work show yeast count colony in co-culture *S. cerevisea* with *C. tropicalis* significantly ($P<0.05$) higher than mono-culture *S. cerevisea* or *C. tropicalis* in the medium with or without phenol. We hypothesized there are synergistic mechanism to stimulate growth yeast through simultaneous utilization of fermentable sugars and degraded phenol in the growth medium by co-culture *S. cerevisiae* with *C. tropicalis*. Some investigators have reported *C. tropicalis* can degrade phenol component **(Krug *et al.*, 1985; Komarkova *et al.*, 2003; Ahuatzi-Chacon *et al.*, 2004; Wang *et al.*, 2012)**. **Jönsson *et al.* (2013)** and **Larsson *et al.* (2000**) reported *S. cerevisiae* can convert some inhibitory phenolics to less toxic compounds such as coniferyl aldehyde is reduced to coniferyl alcohol and dihydroconiferyl alcohol. **Kuntiya *et al.* (2013)** reported phenol can be degraded and used as a source of carbon energy by *C. tropicalis*.

Residue and sugars consumption

Addition of furfural to the growth medium significantly ($p<0.05$) influenced on glucose residue (Fig 3) and xylose (Fig 4), but no significantly ($p>0.05$) influenced on arabinose residue (Fig 5A). Addition of furfural to the growth medium also significantly ($p<0.05$) decreased glucose (Fig 3B) and xylose (Fig 4B) consumption, but no significantly ($p>0.05$) influenced on arabinose consumption (Fig 5B). Glucose, fructose, and mannose are fermented via the Embden-Meyerhof pathway of glycolysis, and galactose requires the Leloir pathway (**Wendland *et al.*, 2009**). Effect inhibitory furfural and phenol on glucose consumption were reported by several researchers (**Lin *et al.*, 2015; Sluiter *et al.*, 2010; Palmqvist *et al.*, 2000, Almeida *et al.*, 2009: Larsson *et al.*, 1999**). **Wikandari *et al.* (2010)** reported consumption glucose by *S. cerevisiae* isolate of Bekonang only 34.94 and 1.93% in the medium containing 1.0 and 1.5 $g.L^{-1}$ of furfural, respectively.

However, this work show glucose consumption by co-culture *S. cerevisiae* with *C. tropicalis* significantly ($P<0.05$) higher than mono-culture *S. cerevisiae* or *C. tropicalis* in the medium with or without furfural. Higher glucose consumption by co-culture than mono-culture in this study suspected due to degradation of furfural by each yeast in the mixture fermentation. Under anaerobic conditions, *S. cerevisiae* can convert furfural to furfuryl alcohol (**Diaz de Villegas *et al.*, 1992; *Sárvári Horváth et al., 2003)*** *and r*eduction of furfural has been linked to the co-factor NADH (**Wahlbom *et al.*, 2002**).

In addition, higher sugar consumption by co-culture than mono-culture in this study also suspected due to the contribution of glucose consumption by *C. tropicalis* in the substrate mixture of glucose and xylose. In this work, *C. tropicalis* can consume glucose from media although lower than *S. cerevisiae*. This observation similar with **Panchal *et al.* (1988)** and **du Preez *et al.* (1986)** who reported a diauxic (sequential) consumption of D-glucose and D-xylose in the same order by *C. shehatae* and *P. stipites* when using mixtures of these sugars in the culture medium. **Laplace *et al.* (1993)** reported co-culture *C. shehatae* with *S. cerevisiae* completely consumed D-glucose from mixture medium containing 70% of D-glucose and 30% of D-xylose after 14 h fermentation,

while D-xylose, in practice, not been consumed. These researchers suggest xylose consumption by *C. shehatae* can be inhibited in the presence of glucose.

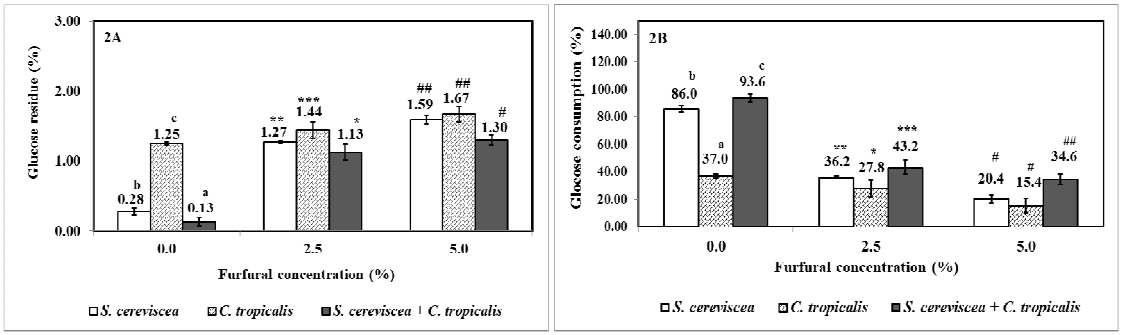

Figure 2 Glucose residue (2A) and consumption of glucose (2B) after five days fermentation by mono and co-culture *S. cerevisiae* with *C. tropicalis* in the media plus different furfural concentration, the values are means of 5 independent observations in same furfural concentration different superscripts ([a] and [b], * and ** or [#], [##] and [###]) differ significantly ($p<0.05$).

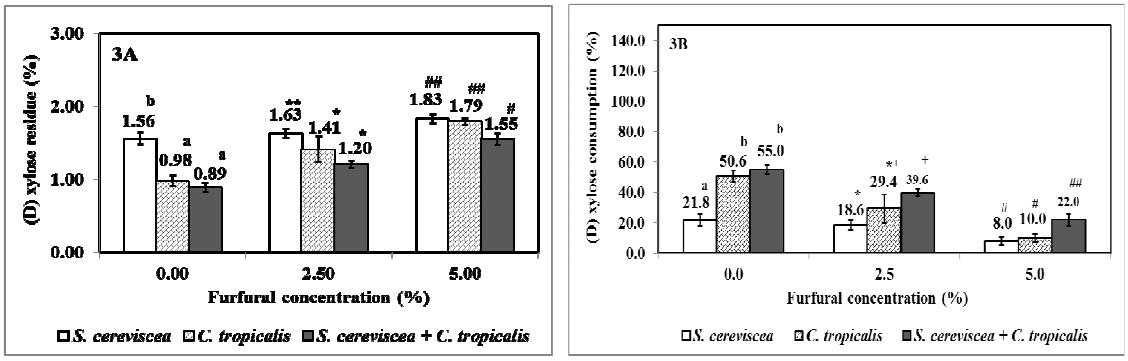

Figure 3 (D) xylose residue (3A) and consumption of xylose (3B) after five days fermentation by mono and co-culture *S. cerevisiae* with *C. tropicalis* in media plus different furfural concentration, the values are means of 5 independent observations in same furfural concentration different superscripts ([a] and [b], * and ** or [#], [##] and [###]) differ significantly ($p<0.05$).

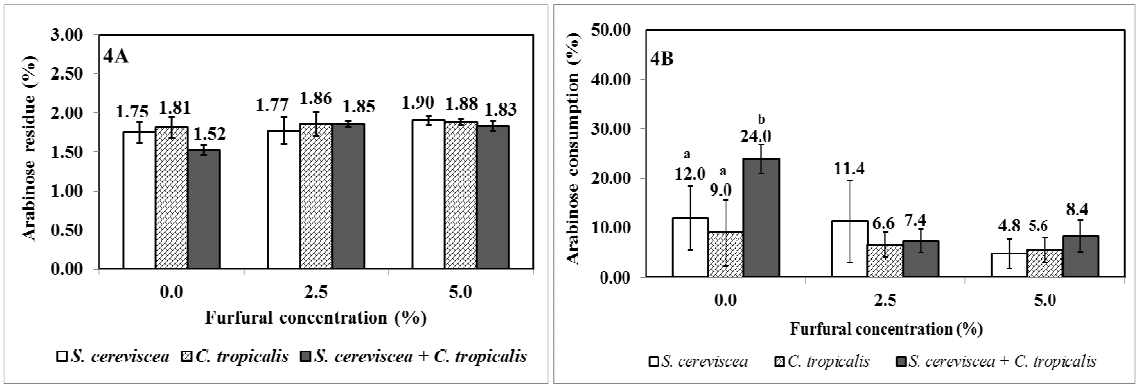

Figure 4 Arabinose residue (4A) and consumption of arabinose (4B) after five days fermentation by mono and co-culture *S. cerevisiae* with *C. tropicalis* in media plus different furfural concentration, the values are means of 5 independent observations in same furfural concentration different superscripts ([a] and [b]) differ significantly ($p<0.05$).

In this work, D(+) xylose consumed by mono-culture *C. tropicalis* and co-culture *S. cerevisiae* with *C. tropicalis* higher than *C. tropicalis*. **Cheng *et al.* (2014)** reported *Candida tropicalis* W103 was able to use xylose as the carbon source for cell growth under aerobic or anaerobic conditions and when glucose was used as the carbon source, ethanol was produced under aerobic or anaerobic

conditions, but *C. tropicalis* grew slightly slower under anaerobic conditions than under aerobic conditions and displayed sequential sugar consumption, first utilizing glucose and then xylose. Higher D(+) xylose consumption by co-culture than mono-culture in this study besides allegedly due to inhibitors degradation by each yeast in the mixture fermentation as describe before, as well as the contribution *S. cerevisiae* to consume D(+) xylose. Native *S. cerevisiae* does not metabolize xylose (**Jeffries and Jin, 2004; Lin and Tanakan, 2006**) and nearly all reported xylose isomerase-based pathways in *S. cerevisiae*suffer from poor ethanol productivity, low xylose consumption rates, and poor cell growth compared with an oxidoreductase pathway and, additionally, often require adaptive strain evolution **(Lee *et al*., 2012).** As all yeasts of the genus *Saccharomyces* lack the gene that produces the enzyme xylose isomerase (**Van Maris *et al*., 2006**), conversion of xylose to xylulose is necessary for carbon uptake (**Chiang *et al*., 1981; Gong *et al*., 1981**). Although low (21.80%), this work indicates that *S. cerevisiae* can consume D(+) xylose, allegedly due to lack of glucose in culture medium, as a mechanism of adaptation to nutritional deficiencies or our *S. cerevisiae* has undergone mutations in fermentation conditions. Figure 5 show the glucose, D(+) xylose and arabinose residues in the media after fermented by *S. cerevisiae*. **Shin *et al.* (2015)** suggested *S. cerevisiae* is able to ferment xylose but first utilizes D-glucose before the D-xylose can be transported and metabolized.

Addition of furfural to the growth medium significantly ($p<0.05$) decreased arabinose consumption by mono or co-culture *S. cerevisiae* with *C. tropicalis* in the fermentation media. Co-culture *S. cerevisiae* with *C. tropicalis* significantly ($p<0.05$) increased arabinose consumption in the fermentation media. **Schimer-Michel *et al.* (2008)** argued that arabinose be metabolize in a later phase, when both glucose and xylose were exhaused. Generally, in this work *S. cerevisiae* and *C. tropicalis* can consume arabinose in the media very low with or without furfural . We found arabinose consumption depends on the availability of glucose and xylose in the media.

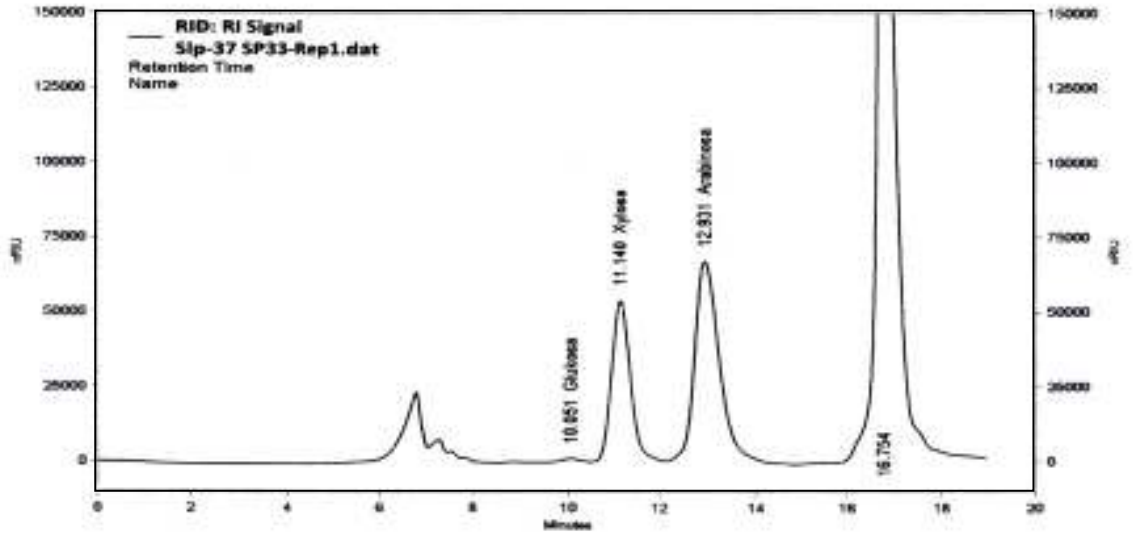

Figure 5. An HPLC chromatogram of residue glucose, D(+) xylose and arabinose in medium after five days fermentation by *S.cerevisiae*.

Addition of phenol to the growth medium significantly ($p<0.05$) influnced glucose (Fig 6A) and xylose (Fig 7A) residue, but no significantly ($p>0.05$) influenced on arabinose residue (Fig 8A). Addition of phenol to the growth medium also significantly ($p<0.05$) decreased glucose (Fig 6B) and xylose (Fig 7B) consumption, but no significantly ($p>0.05$) effect on arabinose consumption (Fig 8B). The toxicity of phenolics is very variable as it depends on the functional groups **(Ando *et al*., 1986; Jonsson *et al*., 2013; Adeboye *et al*., 2014)**, more methoxy groups are related to high hydrophobicity and toxicity **(Klinke *et al*., 2004)**. Yeast *S. cerevisiae* can assimilate many of phenolics which can be part of the detoxification process occurring during fermentation **(Mills *et al*., 1971; Delgenes *et al*., 1996).** Phenolic compounds mainly interfere with proteins function and trigger changes in the protein to lipid ratio **(Keweloh *et al*., 1990)**. Hence, these compounds affect cellular functions like sorting and signaling, as well as cause membrane swelling **(Caspeta *et al.* 2015)**.

Richard *et al.* (2003) have describe the fungal pathways L-arabinose and D-xylose, to convert L-arabinose and D-xylose to D-xylulose 5-phosphate go through oxidation and reduction reactions before they are phosphorylated by xylulokinase. D-Xylose is first reduced to xylitol by an reduced nicotinamide adenine dinucleotide phosphate (NADPH)-consuming reaction. Xylitol is then oxidised by an NADþ-consuming reaction to form D-xylulose. In fungi, L-arabinose goes through four redox reactions. Two oxidations are coupled to NADþ consumption and two reductions to NADPH consumption. Futhermore, **Richard *et al.*** (2003) reported *S. cerevisiae* enables growth on L-arabinose and under anaerobic conditions ethanol is produced from L-arabinose, but at a very low rate. Similar furfural, in this work *S. cerevisiae* and *C. tropicalis* can consume arabinose in the media very low with or without phenol. We found arabinose consumption depends on the availability of glucose and xylose in the media.

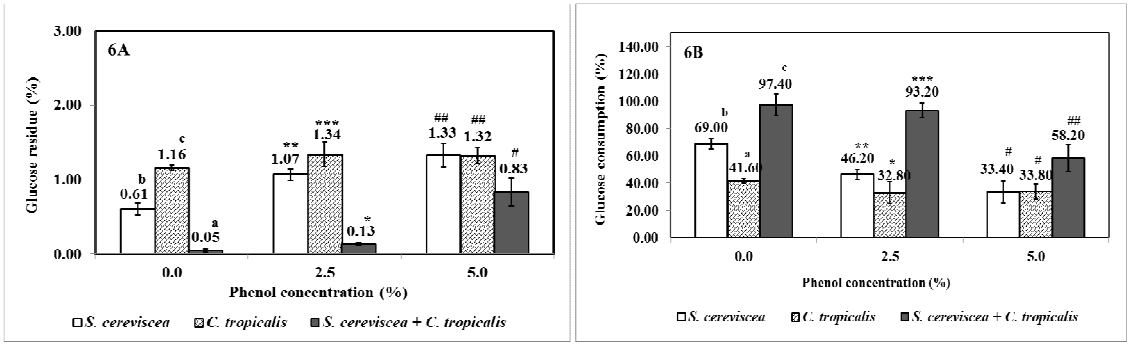

Figure 6 Glucose residue (6A) and consumption of glucose (6B) after five days fermentation by mono and co-culture *S. cerevisiae* with *C. tropicalis* in media plus different phenol concentration, the values are means of 5 independent observations in same phenol concentration different superscripts ([a] and [b], * and ** or [#], [##] and [###]) differ significantly ($p<0.05$).

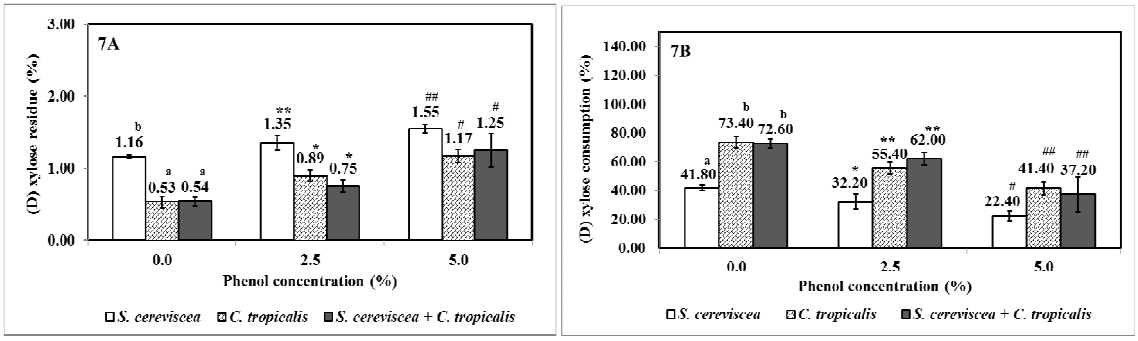

Figure 7 Xylose residue (7A) and consumption of xylose (7B) after five days fermentation by mono and co-culture *S. cerevisiae* with *C. tropicalis* in media plus different phenol concentration, the values are means of 5 independent observations in same phenol concentration different superscripts ([a] and [b], * and ** or [#], [##] and [###]) differ significantly ($p<0.05$).

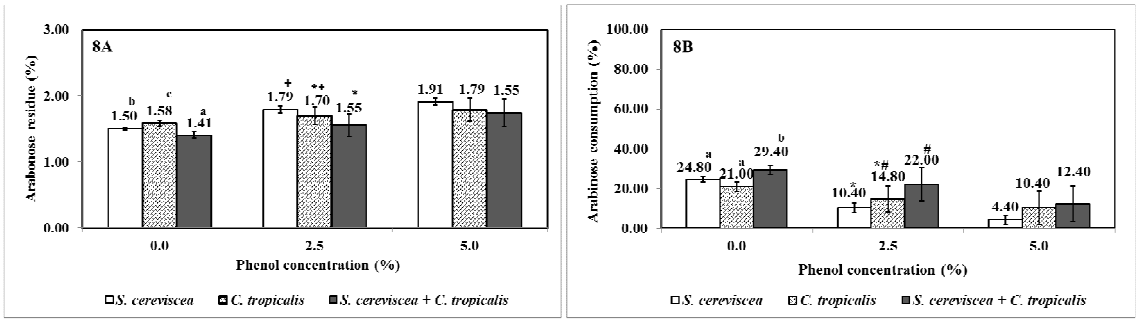

Figure 8 Arabinose residue (8A) and consumption of arabinose (8B) after five days fermentation by mono and co-culture *S. cerevisiae* with *C. tropicalis* in media plus different phenol concentration, the values are means of 5 independent observations in same phenol concentration different superscripts (^{a} and ^{b}, or *, $^{*+}$, and $^{+}$) differ significantly ($p<0.05$).

Ethanol production

Saccharomyces cerevisiae ferments certain sugars very efficiently into ethanol, even under aerobic conditions. Addition of furfural (Fig 9A) or phenol to the growth medium significantly ($p<0.05$) decreased ethanol yields. This work indicated furfural inhibits sugar conversion to ethanol in mono-culture and co-culture *S. cerevisea* with *C. tropicatlis*. Some investigators have been reported inhibitory effect of furfural on yeast growth and ethanol production. **Zaldivar *et al.* (1999)** reported furfural and HMF to compromise membrane integrity leading to extensive membrane disruption/leakage, which eventually will cause reduction in cell replication rate, ATP production, and consequently lower ethanol production. **Agbogbo and Wenger, (2007)** reported furfural concentration 1.5 g.L^{-1} can inhibit respiration and growth of microorganism and leading reduced ethanol production 90.4% and productivity 85.1%. **Ylitervo *et al.* (2013)** reported furfural at the lower concentration (0.8 and 1.5 g L−1) decreases ethanol yields less than 10% and at the higher concentration decrease ethanol yield up to around 20% and 60%.

Phenolic compounds are known to partition into biological membranes altering the permeability and lipid/protein ratio, which thus increases cell fluidity, leading to cell membrane disruption, dissipation of proton/ion gradients and compromising the ability of cellular membranes to act as selective barriers (**Heipieper *et al*., 1994**). **Kuntiya *et al.* (2013)** reported isolate *C. tropicalis* No 10 fully able degradation phenol concentration 100 mg.L^{-1} at 20-42^{o}C, but this degradation inhibited by decreasing concentration of oxygen in media.

However, this work show ethanol yields in the co-culture *S. cerevisea* with *C. tropicalis* significantly ($P<0.05$) higher than mono-culture *S. cerevisea* or *C. tropicalis* with or without inhibitors furfural or phenol in the medium growth. We hypothesized there are synergistic mechanism to stimulate ethanol production through simultaneous utilization of fermentable sugars such as glucose and xylose and degrade furfural or phenol in the growth medium by co-culture *S. cerevisea* with *C. tropicalis.* **Chen (2011)** suggested that co-culture fermentation is a strategy for efficient conversion of glucose and xylose to ethanol and increase ethanol yield and production rate. **Thurnheer *et al.* (1988)** and **Shim *et al.* (2002)** suggested that a co-culture as a mimic of natural environment has been used for biodegradation of aromatic compounds **Bader *et al.* (2010)** suggested that in cocultures, degradation and metabolization of substrates occur by the combined metabolic activity of the known microbial strains under aseptic conditions.Some investigators have been reported ethanol production by co-culture microorganism in the medium containing furfural. **Wan *et al.* (2012)** reported co-culture *S. cerevisiae* Y5 and *P.stipitis* CBS6054 effectively converting glucose and xylose to ethanol, as well as effectively degrading inhibitors in the hydrolysate. Furthermore, **Wan *et al.* (2012)** reported co-culture *S. cerevisiae* Y5 and *P.stipitis* CBS6054 used up and completely metabolized glucose, furfural and 5-HMF within 12 h; xylose was used up in 96 h at 80 rpm with ethanol concentration and yield of 27.4 g L^{-1} and 0.43 g ethanol/g sugar without detoxification of the hydrolysate, respectively. **Komarkova *et al.* (2003)** reported *C. tropicalis* can use phenol concentration 500 mg.L^{-1} as carbon source and energy.

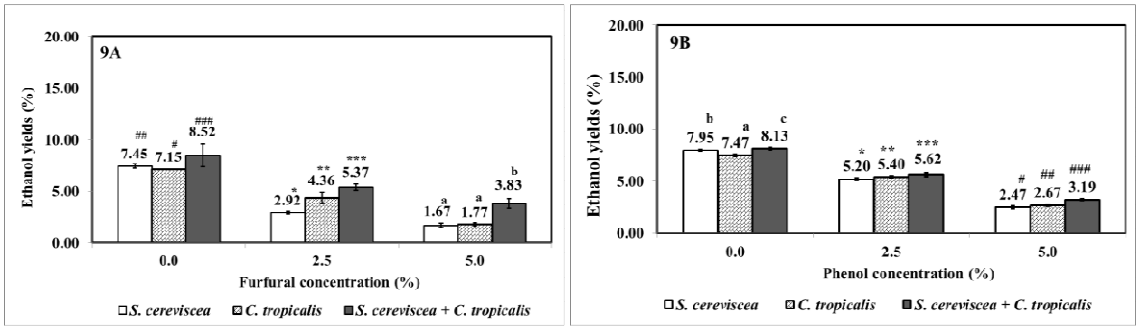

Figure 9 Ethanol yields in media plus different furfural (9A) and phenol (9B) concentration after five days fermentation by mono and co-culture *S. cerevisiae* with *C. tropicalis,* the values are means of 5 independent observations in same furfural or concentration different superscripts (^{a} and ^{b}, *,** and *** or $^{\#}$, $^{\#\#}$ and $^{\#\#\#}$) differ significantly ($p<0.05$).

Efficiency fermentation

Addition of furfural (Fig 10A) and phenol (Fig 10B) to the growth medium significantly ($p<0.05$) decreased efficiency fermentation of ethanol production by mono and co-culture *S. cerevisiae* with *C. tropicalis*. However, co-culture *S. cerevisiae* with *C. tropicalis* significantly ($p<0.05$) higher efficiency fermentation of ethanol production than mono-culture *S. cerevisiae* or *C. tropicalis* from medium with or without furfural and phenol. Although *S. cerevisiae* efficiently convert hexoses into ethanol, but this native yeast is not able to metabolize pentose sugars present in lignocellulosic hydrolyzate. This work indicates that co-culture of *S. cerevisiae* and *C. tropicalis* more efficient use of sugar in media to be converted into ethanol. Co-culture *S. cerevisiae* with *C. tropicalis* higher consume glucose and D(+) xylose than *C. tropicalis* and *S. cerevisiae* alone. **Wang *et al*. (2012)** suggested that the co-culture between the two microorganisms in a single process is an alternative to reduce the effects of inhibitors present in the media. **Hickert *et al*. (2012)** reported co-culture *C. shehatae* HM 52.2 and *S. cerevisiae* ICV D254 can produce ethanol 0.42 from synthetic media and 0.51 from rice husk hydrolysate. **N'Guessan *et al*. (2010)** reported that ethanol production from sorghum by co-culture *C. tropicalis* and *S. cerevisiae* with a 2: 1 ratio is higher than pure cultures of *S. cerevisiae*. Co-cultures of *S. cerevisiae* is more preferred hexose sugars with yeast more preferred pentose to produce ethanol efficiently is one alternative for optimizing the production of ethanol in the hydrolyzate containing xylose (**Gutiérrez-Rivera *et al*., 2012; Karagoz and Ozkan, 2014; Licht, 2006**). **Gutiérrez-Rivera *et al*. (2012)** reported ethanol production by co-culture *S. cerevisiae* ITV-01 and *Pichia stipitis* NRRL Y-7124, 5 times higher than ethanol production by mono-culture *S. cerevisiae* ITV-01 and mono-culture *P. stipitis* NRRL Y-7124. Increased ethanol productivity can be caused enrichment substrates that can be utilized as *S. cerevisiae* use six carbon sugars (glucose) and *P. stipitis* using 5-carbon sugar to produce ethanol **(Tesfaw and Assefa, 2014)**. Co-culture fermentation *S. cerevisiae* MTCC 174 with *Scheffersomyces stipitis* NCIM No. 3497 can produce maximum ethanol (20.8 $g.l^{-1}$) higher than ethanol production by mono-culture *S. cerevisiae* MTCC 174 (14.0 $g.l^{-1}$) or *S. stipitis* NCIM No. 3497 (12.2 $g.l^{-1}$) **(Singh *et al*., 2014)**. Ethanol production by co-culture *S. cerevisiae* ATCC 26602 and *P. stipitis* DSM 3651 (7.36 $g.l^{-1}$) higher than mono-culture *S. cerevisiae* (6.68 $g.l^{-1}$) from wheat straw media with pre-treatment H_2O_2 and enzyme hydrolysis **(Karagoz and Ozkan, 2014)**. Tolerance microorganisms including co-culture fermentation to inhibitors and ethanol is one of the problems the production of ethanol from lignocellulosic waste. **Gutiérrez- Rivera *et al*. (2012)** reported *P. stipitis* NRRL Y-7124 has a low tolerance to ethanol that produced by *S. cerevisiae* ITV-01 and prevent further ethanol production by *P. stipitis* NRRL Y-7124. This work shown co-culture *S. cerevisiae* and *C. tropicalis* have high tolerance to inhibitors and higher ethanol yield than mono-culture *S. cerevisiae* or mono-culture *C. tropicalis* in basal medium and basal medium plus furfural or phenol. Co-culture *S. cerevisiae* and *C. tropicalis* also showed higher fermentation efficiency than mono-culture in basal media and basal media plus furfural or phenol. Increased ethanol production and efficiency of co-culture fermentation was allegedly due to the contribution of *C. tropicalis* to convert xylose into ethanol. **Karagoz and Ozkan (2014)** suggested that increased ethanol production by co-culture *S. cerevisiae* and *P. stipitis* due to the contribution of *P. stipitis* to convert xylose into ethanol. **Hickert *et al*. (2013)** reported co-culture *C. shehatae* HM 52 with *S. cerevisiae* ICV D254 in synthetic medium and rice hull hydrolyzate effectively convert glucose and xylose simultaneously, maximizing the utilization rate of the substrate, increasing the yield and rate of ethanol production.

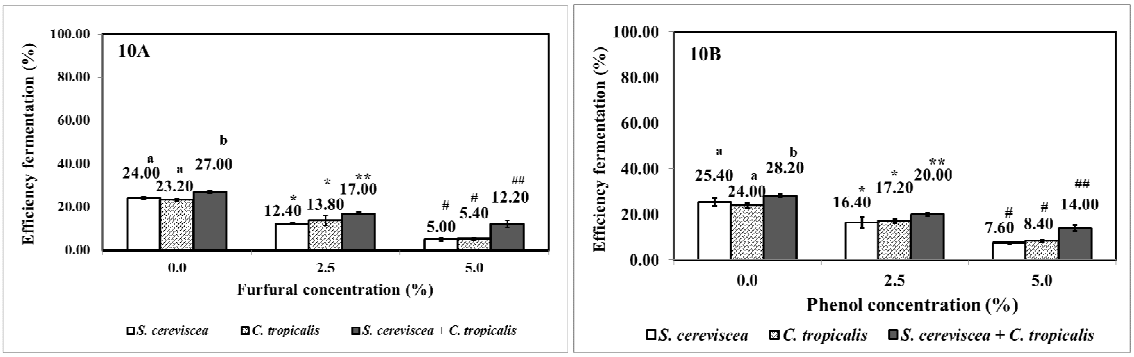

Figure 10 Efficiency fermentation ethanol production by mono and co-culture *S. cerevisiae* with *C. tropicalis* from basal media plus furfural (10A) and phenol (10B) with different concentration.

CONCLUSIONS

Sugar consumption and ethanol production by co-culture *S. cerevisiae* with *C.tropicalis* in the media fermentation with or without inhibitors have higher than mono *S. cerevisiae* or *C.tropicalis*. Co-culture *S. cerevisiae* with *C.tropicalis* demonstrated high tolerance to inhibitors fermentation than mono-cultures for ethanol production. Glucose and xylose consumption by *S. cerevisiae* and *C.tropicalis* contribute to the improvement and efficiency of ethanol production by culture fermentation from mixture substrate. Co-culture *S. cerevisiae* FNCC 3012 with *C.tropicalis* FNCC 3033 demonstrated potential as a fermentation process for ethanol production from lignocelulosic medium or media content inhibitors. The use of this co-culture effectively utilize hexose and pentose sugars in the substrate, increasing the yield and efficiency of fermentation ethanol production.

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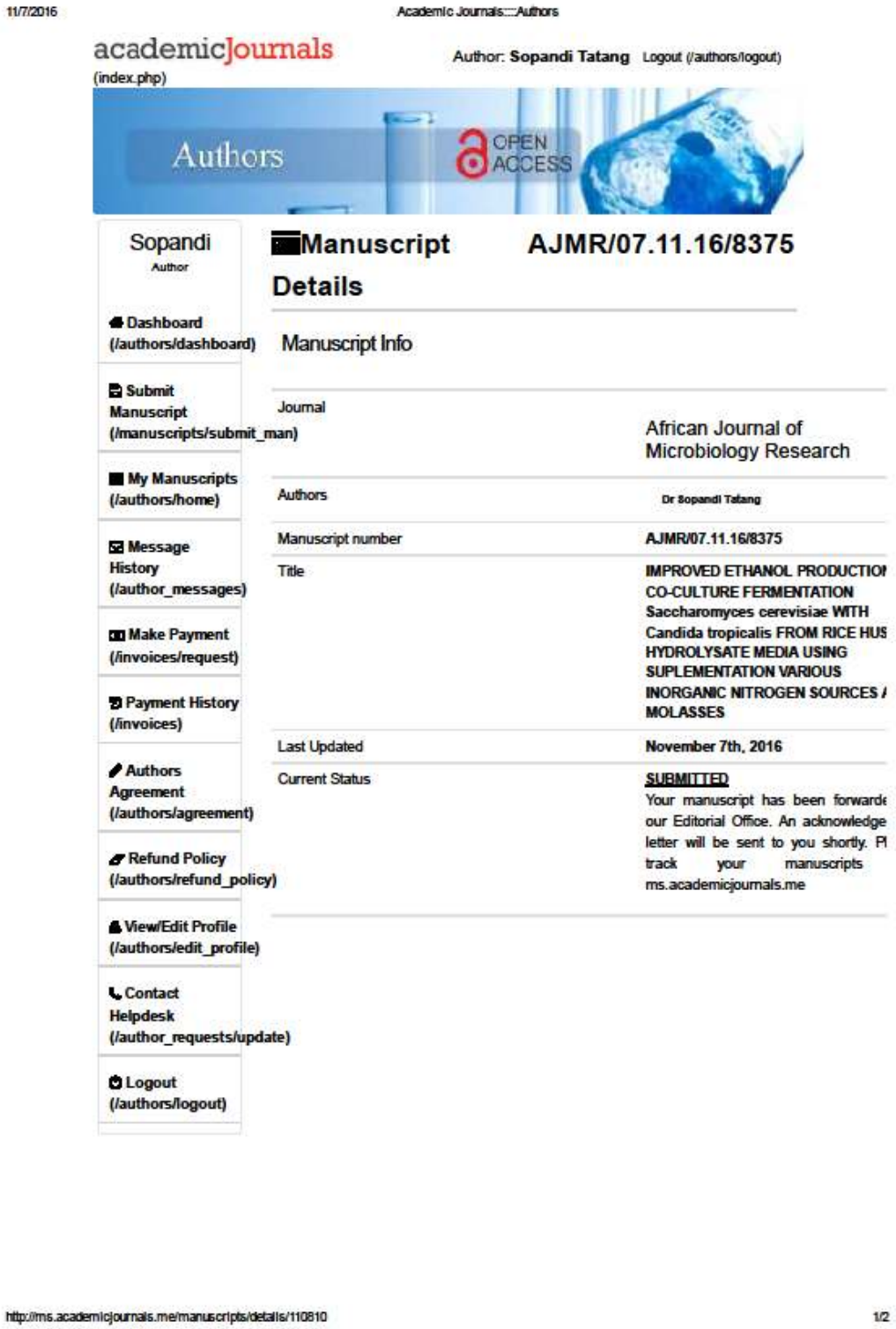

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IMPROVED ETHANOL PRODUCTION BY CO-CULTURE FERMENTATION *Saccharomyces cerevisiae* WITH *Candida tropicalis* FROM RICE HUSK HYDROLYSATE MEDIA USING SUPLEMENTATION VARIOUS INORGANIC NITROGEN SOURCES AND MOLASSES

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ABSTRACT

The use of agricultural by-product as feed stock and co-culture fermentation is a good strategy to improve efficiency and ethanol production. Almost rice husk have low protein and nitrogen content and need to be supplemented with nitrogen for fermentation process. This research sought to determine optimal supplementation nitrogen sources and molasses to rice husk stream–based fermentation medium, initial pH and incubation time to maximize ethanol production by co-culture *S. cerevisiae* with *C. tropicalis.* Urea, sodium nitrate and ammonium nitrate were used nitrogen sources and molasses was used carbon sources. Co-culture *S. cerevisiae* with *C. tropicalis* can use different nitrogen sources and molasses for growth and ethanol production. Molasses supplementation to the rice husk hydrolysate medium, initial pH and incubation period significant influenced on ethanol yield and content of nitrogen and carbon DDG. Maximum ethanol yield (20.32± 0.42%) with nitrogen (4.40± 0.11%) and carbon (9.20± 1.01%) content of DDG were obtained in the rice husk hydrolysate medium contain 16.0 g/l urea, 12.0 g/l $NaNO_3$, 12.0 g/l NH_4NO_3, 1.0 g/l KH_2PO_4, 0.7 g/l $MgSO_4 \cdot 7H_2O$, 20 ml/l molasses, 1.0 g/l KH_2PO_4 and 0.7 g/l $MgSO_4 \cdot 7H_2O$ with initial pH 5.5 and 6 days incubation period at 28-29^oC, 50% relative humidity in the dark for 5 d in a rotary incubator at 60 rpm.

Key words: rice husk, *Saccharomyces cerevisiae, Candida tropicalis,* co-culture, ethanol yield, nitrogen and molasses

INTRODUCTION

The use of agricultural by-product as feed stock and co-culture fermentation is a good strategy to improve efficiency and ethanol production. Lignocellulosic biomass include agricultural by-product has been considered as possible raw material for ethanol

production due to its renewability, large quantities, relatively low prices than grain or sugar, potential environmental benefits and does not compete with food (Cardona and Sa´nchez, 2007; Kumar et al., 2008; Lee and Huang, 2000; Mielenz, 2001; Service, 2007; Zaldivar et al,. 2001; Ishola and Taherzadeeh, 2014). The lack of a microorganism able to ferment efficiently all sugars released by hydrolysis from lignocellulosic materials has been one of the main factors preventing utilization of lignocellulose (Zaldivar et al., 2001). *Saccharomyces cerevisiae*, which is by far the dominant yeast used for ethanol production, naturally converts glucose to ethanol but does not metabolize xylose (Jeffries and Jin, 2004; Lin and Tanakan, 2006).

Although it varies, almost rice husk have low protein and nitrogen content and need to be supplemented with nitrogen for fermentation process. Crude protein and nitrogen rice husk respectively 4.38% and 0.7% with the C / N ratio 57.93 (Ofoefule et al., 2011). Nitrogen sources such as ammonium (Jones et al., 1994 and Srichuwong et al., 2009) and urea (Jones and Ingledew, 1994; Yue et al., 2010) are widely used to increase yeast growth, rate of sugar utilize and reduce fermentation time (Chniti et al., 2015). Urea not only promoted the specific growth rate and ability of ethanol tolerance, but also increased the ethanol yield and reduced the formation of by product (Yue et al., 2010). However, several investigators have been reported negative effects of using ammonium and urea as nitrogen supplements in ethanol fermentation (Laopaiboon et al., 2009; Wang et al., 2003; Beltran et al., 2005; Chniti et al., 2015).

Elements such as carbon and nitrogen are both required in yeast metabolism. The type and concentration of carbon and nitrogen sources, as well as the C/N ratio of the medium for *S. cerevisiae* cultivation, influence cellular growth and metabolites biosynthesis (Thomas et al., 1996). Molasses is a waste of the sugar industry can be used as a substrate for ethanol production by *S.cerevisiae* (Fern´andez-L´opez et al., 2012; Sadik and Halema, 2014). Molasses contains readily utilizable carbohydrates available in the form of fermentable sugars and can be used by the alcohol producing yeasts without any pretreatment (Murtagh, 1999).

The co-culture is a potential bioprocess if there are no cross-interactions among microorganisms, and each microorganism metabolizing its substrate is unaffected by the presence of other microorganism (Park et al., 2012). Co-culture of *S. cerevisiae* and

other microorganism increased ethanol productivity might be due to enhanced substrate utilization (Tesfaw and Assefa, 2014). Co-culture *S. cerevisiae* with other microbes reduces inhibitory compounds in lignocellulosic hydrolysates (Tom´as et al., 2013; Taherzadeh and Karimi, 2011; Wan et al., 2012) increases ethanol yield and production rate (Singh et al., 2014 and Wan, 2012), shortens fermentation time, and reduces process cost (Hickert et al., 2013; Tesfaw and Assefa, 2014). Co-culture *S.cerevisiae* with *C. tropicalis* have ability to generate and convert fermentable sugars from a waste stream rice husk to ethanol (Sopandi and Wardah, 2015). This work explored supplementation of inorganic nitrogen sources and molasses to improve ethanol production by co-culture *S. cerevisiae* with *C. tropicalis* from rice husk hydrolisate.

MATERIAL AND METHODS

Culture microorganism

Saccharomyces cerevisiae FNCC 3012 and *Candida tropicalis* FNCC 3033 were obtained from Microbiology Laboratories, PPAU Gadjah Mada University, Yogyakarta, Indonesia. Saubroad agar (Oxoid) was used to maintain the strain *S. cerevisiae* and *C. tropicalis*. Working stock cultures were prepared from stock, 7 days, 28^{o}C SA plate cultures subcultures from the master stock. Colonies were aseptically sampled by scraping the top with an inoculating loop and transfer to 10 ml sterile water. Inoculums stock suspension were prepared from working stock and diluted to 1.7 x 10^{6} cell/ml, as enumerated using a haemocytometer.

Rice husk hydrolysis

Locally farm-sourced rice husk from Sidoarjo, Indonesia, were air-dried then ground to approximately 2-mm diameter particles using a mill. The rice husk milled (900 g) were steamed at 130^{o}C for 3 h, cooled to room temperature, mixed with 15 l, 2.5% H_2SO_4 and autoclaved for 15 min at 121^{o}C. Hydrolysate was cooled and stored at 1-5^{o}C in the dark until used.

Inorganic nitrogen supplementation

The basal medium contained 1500 ml rice husk hydrolysate, 1.0 g/l KH_2PO_4, and 0.7 g/l $MgSO_4.7H_2O$ was dispensed into three 1000 ml Erlenmeyer flasks and added urea, sodium nitrate ($NaNO_3$) and ammonium nitrate (NH_4NO_3) to final individual

concentrations of 9.0 g/l, respectively. Media were mixed thoroughly, adjusted pH 5.5 with add HCl 1 N, autoclaved, cooled to room temperature. One hundred millilitre was then aseptically dispensed into individual Erlenmeyer flasks (250 ml) and individually inoculated with one ml of *S. cerevisiae* FNCC 3012 and one ml of *C. tropicalis* FNCC 3033 inoculum stock suspension and all flask incubated at 28-29^{o}C, 50% relative humidity in the dark for 5 d in a rotary incubator at 60 rpm.

Molasses supplementation

Rice husk hydrolysate basal medium (2500 ml) containing 3.0 g/l urea, 3.0 g/l $NaNO_3$, 3.0 g/l NH_4NO, 1.0 g/l KH_2PO_4 and 0.7 g/l $MgSO_4 \cdot 7H_2O$ was dispensed into five 1000 ml Erlenmeyer flasks, added molasses to final concentrations of 0.0,5.0, 10.0,15.0 and 20.0 ml/l. Media were mixed thoroughly, adjusted pH 5.5 with add HCl 1 N, autoclaved and cooled to room temperature. One hundred millilitre was then aseptically dispensed into individual Erlenmeyer flasks (250 ml), one ml of *S. cerevisiae* FNCC 3012 and one ml of *C.tropicalis* FNCC 3033 inoculum stock suspension and all flasks incubated as described above.

Formulation rice husk hydrolysate

Four formulation of rice husk hydolysate media were examined to improve ethanol production by co-culture *S. cerevisiae* FNCC 3012 with *C.tropicalis* FNCC 3033. Rice husk hydrolysate basal medium (2000 ml) containing 1.0 g/l KH_2PO_4 and 0.7 g/l $MgSO_4 \cdot 7H_2O$ was dispensed into four 1000 ml Erlenmeyer flasks. Individually was added 4.0 g/l urea, 3.0 g/l $NaNO_3$, 3.0 g/l NH_4NO_3, and 20 ml/l molasses (F_1), 8.0 g/l urea, 6.0 g/l $NaNO_3$, 6.0 g/l NH_4NO_3, and 20 ml/l molasses (F_2), 12.0 g/l urea, 9.0 g/l $NaNO_3$, 9.0 g/l NH_4NO_3, and 20 ml/l molasses (F_3), and 16.0 g/l urea, 12.0 g/l $NaNO_3$, 12.0 g/l NH_4NO_3 and 20 ml/l molasses (F_4). Media were mixed thoroughly, adjusted pH 5.5 with add HCl 1 N, autoclaved and cooled to room temperature. One hundred millilitres was then aseptically dispensed into individual Erlenmeyer flasks (250 ml), one ml of *S. cerevisiae* FNCC 3012 and one ml of *C.tropicalis* FNCC 3033 inoculum stock suspension and all flasks incubated as described above.

Initial medium pH

To examine the effect of initial medium pH, 100 ml rice husk hydrolysate basal medium containing 1.0 g/l KH_2PO_4, 0.7 g/l $MgSO_4 \cdot 7H_2O$, 16.0 g/l urea, 12.0 g/l $NaNO_3$, 12.0

g/l NH_4NO_3 and 20 ml/l molasses was aliquoted into 8, 250-ml Erlenmeyer flasks and the pH of each adjusted to 3.5, 4.0, 5.4.5, 5.0, 5.5, 6.0, 6.5, and 7.0 prior to autoclaving. After cooling to room temperature, flasks were inoculated with 1-ml *S. cerevisiae* FNCC 3012 and 1- ml of *C.tropicalis* FNCC 3033 inoculum stock suspension and incubated as described above.

Incubation period

Effect of incubation period on ethanol yield, nitrogen and carbon content destilate residue was examined using a rice husk hydrolysate basal medium containing 1.0 g/l KH_2PO_4, 0.7 g/l $MgSO_4 \cdot 7H_2O$, 16.0 g/l urea, 12.0 g/l $NaNO_3$, 12.0 g/l NH_4NO_3 and 20 ml/l molasses. Erlenmeyer flasks (250 ml) containing 100-ml sterile medium were inoculated with 1-ml *S. cerevisiae* FNCC 3012 and 1- ml of *C.tropicalis* FNCC 3033 inoculum stock suspension and incubated as described above. Ethanol yield, nitrogen and carbon content distillate residue analyses were carried out every day up to 9-days incubation. All data presented are means of four simultaneously incubated fermentation culture replicates.

Determination of ethanol

Ethanol were measured using a gas chromatograph Carbomax t70-10-0 column, FID t220 detector, helium as carrier gas with flow rate 40.3 mL/min, tin column Porapack Q, detector temperature at 160^{o}C and column temperature at 180^{o}C with injection volume 1.0 µL. Fermented media were filtered through Whatmann No.1 paper.

Determination of distillers dried grains

To determine distillers' dried grains, whole flask cultures were distillated at 78^{o}C for 60 min and residue poured through predried (100°C) preweighed Whatman No.1 filter paper. Retained material was washed with distilled water then ethanol until colourless and dried at 100°C to constant weight (48 h).

Determination of organic carbon

Levels of total organic carbon (TOC) were determined using the wet oxidation method of Walkey and Black (1965). One hundred millilitre of liquid culture was evaporated at 100°C for approximately 2 h to obtain a dried powder, 0.5 g of which was used for TOC determination.

Nitrogen determination

Nitrogen (NH4-N) concentration was determined using the method of the American Society of Agronomy and Soil Science Society of America (1982). Ten-millilitre culture medium was evaporated at 100°C for approximately 2 h to obtain a dried powder. Samples (50 mg) were added to digestion tubes and 1-g selenium mixture (mashed 1.55 g $CuSO_4$, 96.9 g Na_2SO_4 and 1.55 g selenium) and 3-ml 97% H_2SO_4 added, mixed and digested at 350°C for 4 h to obtain a colourless extract, cooled to room temperature, diluted to 50 ml with distilled water, shaken vigorously and left to stand overnight. Two-millilitre of extract was placed, transferred to a new borosilicate glass test tube and 4-ml tartrate buffer (50 g NaOH and 50 g $KNaC_4H_4O_6$ in 1000 ml distillated water) and sodium phenate solution (100 g NaOH and 125 g phenol in 1000 ml distillated water) successively added, mixed and let stand for 10 min. Four-millilitre, 5% NaOCl was then added, the mixture shaken, let stand for 10 min and absorbance measured at 636 nm. $(NH4)_2SO_4$ was used to prepare N standards.

Statistical analysis

Tukey's honestly significant difference multiple comparison tests were used to segregate significantly different treatments using SPSS 16 software. Analysis of variance (ANOVA) was performed to determine significant differences between experiments ($P < 0.05$).

RESULTS

Effect inorganic nitrogen supplementation

No significant ($P>0.05$) difference in ethanol yield or nitrogen and carbon content of distillers' dried grains (DDG) was observed between nitrogen source type (Fig. 1). Not significantly ($P > 0.05$) different between ethanol yield from rice husk hydrolysate basal media with supplementation urea (5.15 ± 0.42%), $NaNO_3$ (4.94 ± 0.34%) and NH_4NO_3 (4.88± 0.28%). A similar trend was observed for nitrogen content in DDG, not significantly ($P > 0.05$) different between urea (2.21± 0.27%), $NaNO_3$ (2.41± 0.34%) and NH_4NO_3 (2.39± 0.30%). Addition of inorganic nitrogen to the rice husk hydrolysate basal medium also did significant ($P > 0.05$) on carbon content of DDG, not

significantly (P > 0.05) different between urea (13.57± 2.90%), $NaNO_3$ (10.53± 1.79%) and NH_4NO_3 (11.64± 2.83%).

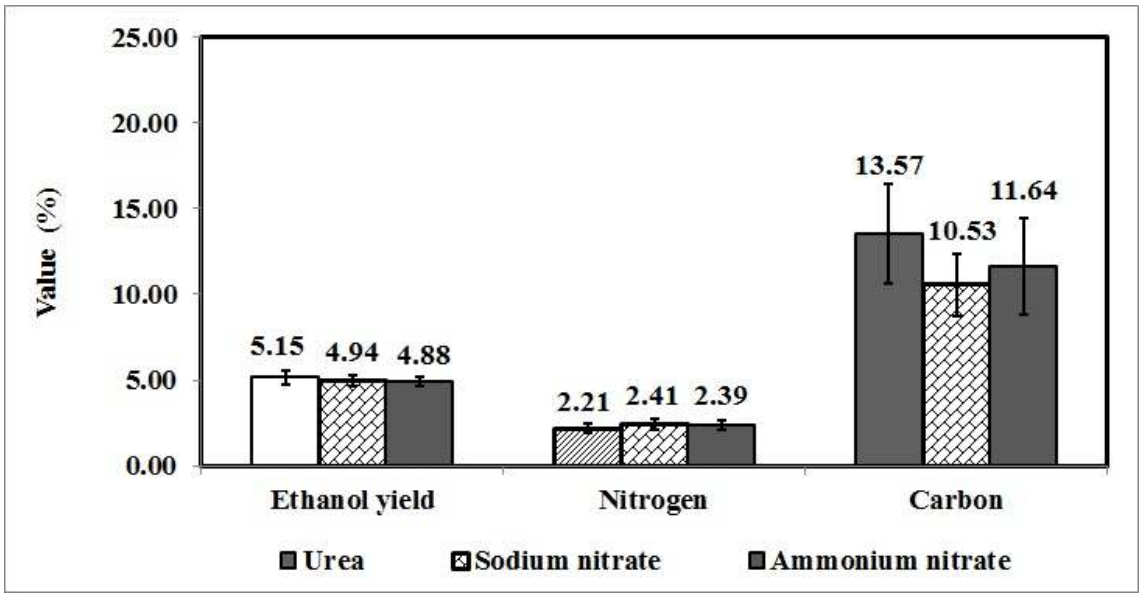


Figure 1. Ethanol yield, nitrogen and carbon content in DDG from rice husk hydrolysate medium supplemented inorganic nitrogen different that fermented by co-culture *S. cerevisiae* with *C. tropicalis*.

Effect molasses supplementation

Addition of 5–20 ml/l molasses to the rice husk hydrolysate basal medium significantly (P<0.05) increased ethanol yield, nitrogen and carbon content of DDG (Fig. 2). Increasing amounts of molasses (5, 10, 15 and 20 ml/l) in the medium progressively increased ethanol yield. Ethanol yield in the basal medium alone (5.38 ±0.28%) significantly (P<0.05) lower than that in the basal medium plus 5 ml/l (6.86± 0.52%), 10 ml/l (8.57± 0.31%), 15 ml/l (9.49± 0.29%), and 20 ml/l molasses (9.75± 0.14%), but no significant (P>0.05) different between 15 ml/l and 20 ml/l molasses. Nitrogen content of DDG from the rice husk hydrolysate basal medium was also significantly (P < 0.05) lower than that in the rice husk hydrolysate basal medium plus molasses (10, 15, and 20 ml/l). Nitrogen content of DDG in the basal medium (2.57± 0.12%) not significant (P>0.05) between in the basal medium plus 5 ml/l molasses ((2.84±0.07%), but significantly (P<0.05) lower than that in basal medium plus 10 ml/l (3.06±0.05%), 15 ml/l (3.45 ±0.28%) and 20 ml/l molasses (3.72±0.06%). However, no significant (P>0.05) different between 15 ml/l and 20 ml/l. This indicated a molasses-concentration

dependent stimulation growth of yeast and ethanol production. While mean, carbon content of DDG in the basal medium (7.90±0,73%) not significant (P>0.05) between in basal medium plus 5 ml/l molasses (9.76± 0.85%), but significantly (P<0.05) lower than that in the basal medium plus 10 ml/l (11.26± 0.60%), 15 ml/l (13.28± 0.99%) and 20 ml/l molasses (13.88± 1.52%). However, no significant (P>0.05) different between 15 ml/l and 20 ml/l.

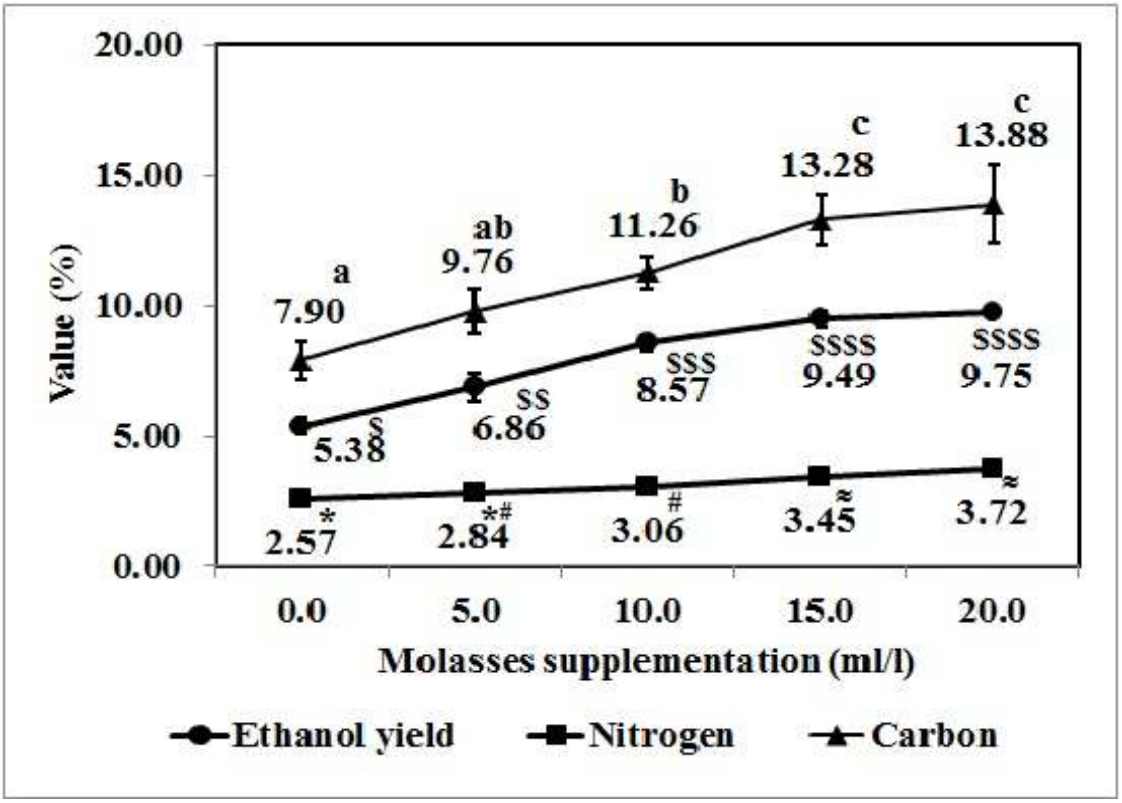
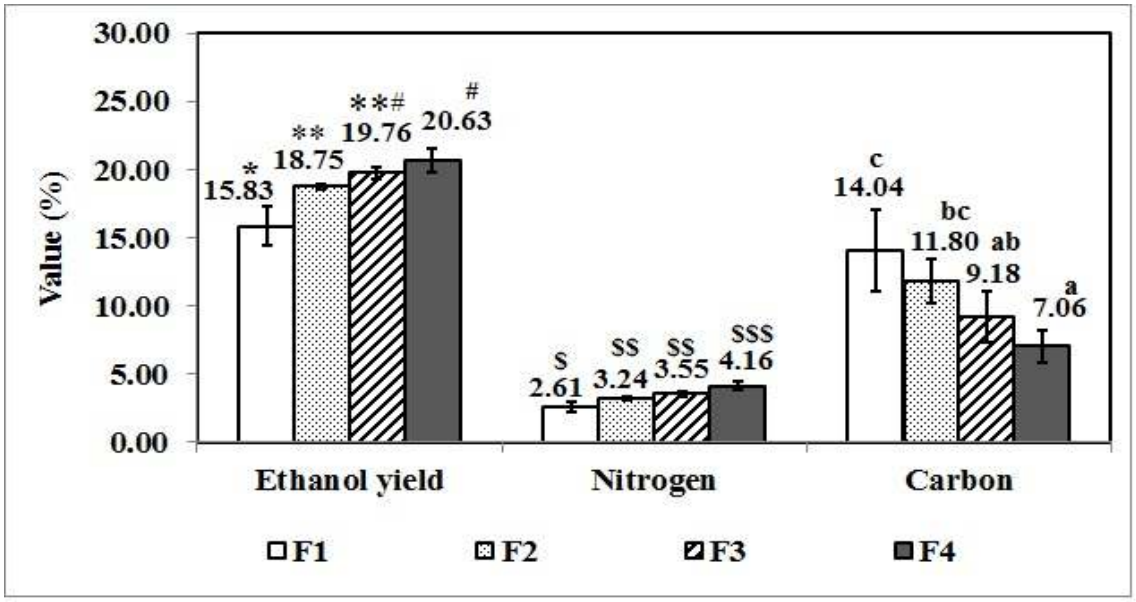


Figure 2 Effect of molasses addition on ethanol yield, nitrogen and carbon content of DDG rice husk hydrolysate medium that fermented by co-culture by co-culture *S. cerevisiae* with *C. tropicalis*.

Formulation rice husk hydrolysate media

Formulation of rice husk hydrolysate media supplemented inorganic nitrogen and molasses significantly (P<0.05) influenced on ethanol yield, nitrogen and carbon content of DDG (Fig.3). Maximum ethanol yield, nitrogen and carbon content of DDG were obtained in the rice husk hydrolysate medium supplemented with 16.0 g/l urea, 12.0 g/l $NaNO_3$, 12.0 g/l NH_4NO_3, 1.0 g/l KH_2PO_4, 0.7 g/l $MgSO_4 \cdot 7H_2O$, 20 ml/l molasses. Ethanol in F1 (the rice husk hydrolysate medium supplemented with 4.0 g/l

urea, 3.0 g/l $NaNO_3$, 3.0 g/l NH_4NO_3, 1.0 g/l KH_2PO_4, 0.7 g/l $MgSO_4 \cdot 7H_2O$, 20 ml/l molasses (15.83± 1.43%) was significantly ($P<0.05$) lower than that in F2 (the rice husk hydrolysate medium supplemented with 8.0 g/l urea, 6.0 g/l $NaNO_3$, 6.0 g/l NH_4NO_3, 1.0 g/l KH_2PO_4, 0.7 g/l $MgSO_4 \cdot 7H_2O$, 20 ml/l molasses (18,75± 0,23%), F3 (the rice husk hydrolysate medium supplemented with 12.0 g/l urea, 9.0 g/l $NaNO_3$, 9.0 g/l NH_4NO_3, 1.0 g/l KH_2PO_4, 0.7 g/l $MgSO_4 \cdot 7H_2O$, 20 ml/l molasses (19.76±0.47%), and F4 (the rice husk hydrolysate medium supplemented with 16.0 g/l urea, 12.0 g/l $NaNO_3$, 12.0 g/l NH_4NO_3, 1.0 g/l KH_2PO_4, 0.7 g/l $MgSO_4 \cdot 7H_2O$, 20 ml/l molasses (20.63± 0.89%), not significantly ($P > 0.05$) different between F2 and F2 and F3.



F1; 1000 ml rice husk hydrolysate, 4.0 g/l urea, 3.0 g/l $NaNO_3$, 3.0 g/l NH_4NO_3, 1.0 g/l KH_2PO_4, 0.7 g/l $MgSO_4 \cdot 7H_2O$, 20 ml/l molasses; F2, 1000 ml rice husk hydrolysate, 8.0 g/l urea, 6.0 g/l $NaNO_3$, 6.0 g/l NH_4NO_3, 1.0 g/l KH_2PO_4, 0.7 g/l $MgSO_4 \cdot 7H_2O$, 20 ml/l molasses; F3, 1000 ml rice husk hydrolysate 12.0 g/l urea, 9.0 g/l $NaNO_3$, 9.0 g/l NH_4NO_3, 1.0 g/l KH_2PO_4, 0.7 g/l $MgSO_4 \cdot 7H_2O$, 20 ml/l molasses; F4, 1000 ml rice husk hydrolysate, 16.0 g/l urea, 12.0 g/l $NaNO_3$, 12.0 g/l NH_4NO_3, 1.0 g/l KH_2PO_4, 0.7 g/l $MgSO_4 \cdot 7H_2O$, 20 ml/l molasses

Figure 3. Effect of different formulation of rice husk hydrolysate culture medium on ethanol yield, nitrogen and carbon content of DDG were fermented by co-culture *S. cerevisiae* with *C. tropicalis*.

While mean nitrogen content of DDG in the F1 medium (2.61±0.34%) significantly ($P<0.05$) lower than that in the F2 (3.24±0.15%), F3 (3.55±0.24%) and F4 medium (7.06± 1.21%), but no significant ($P > 0.05$) difference between F2 and F3 medium. Carbon content of DDG in the F1 medium (14.04± 2.97% was also significantly ($P <$

0.05) higher than that in the F3 (9.18± 1.89%) and F4 (7.06± 1.21%) medium, but no significant (P>0.05) different between F1 and F2 and no significant (P>0.05) different also was observed between F3 and F4 medium.

Effect of initial medium pH

Initial medium pH significantly (P<0.05) affected ethanol yield, nitrogen and carbon content of DDG (Fig.5). In this works shows *S. cerevisiae* and *C. tropicalis* grew and produced ethanol in co-culture over a broad pH range (3.0-7.0). An initial medium pH outside 5.5–6.5 decreased ethanol yield, nitrogen and carbon content of DDG. Ethanol yield at pH 5.5 (20.52± 0.60%) and 6.0 (19.66± 0.16%) was significantly (P<0.05) higher than that at pH 3.0 (1.63 ± 0.31%), 3.5 (2.37± 0.36%), 4.0 (8.25± 0.26%), 4.5 (9.23± 0.26%), 5.0 (13.20± 0.89%), 6.5 (19.10± 0.58%) or 7.0 (11.78±0.50%), with no significant (P > 0.05) difference were observed between pH 5.5 and 6.0 and between pH 6.0 and 6.5. Nitrogen content of DDG pH 5.5 (4.45±0.12%), 6.0 (4.42±0.13%) and 6.5

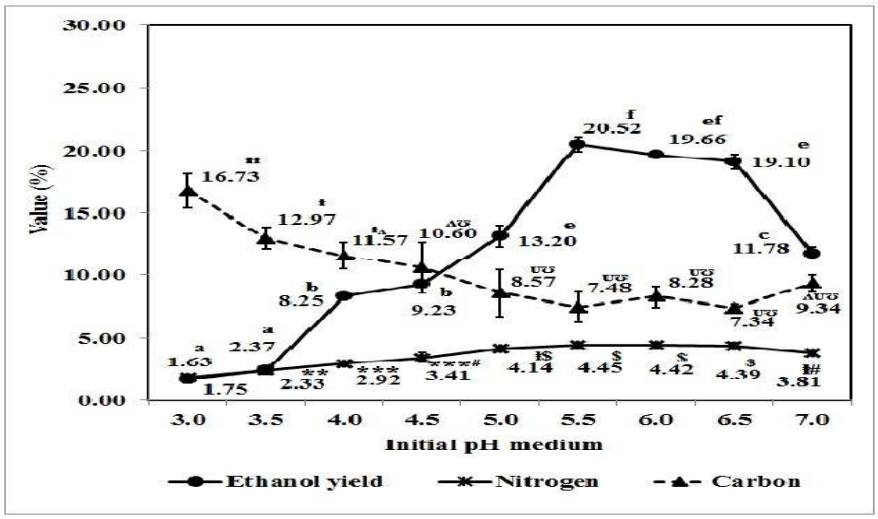


Figure 5. Effect of initial pH medium on ethanol yield, nitrogen and carbon content of DDG from rice husk hydrolysate culture medium with supplemented and fermented by co-culture *S. cerevisiae* and *C. tropicalis*

(4.39±0.26%) was significantly (P<0.05) higher than that at pH 3.5 (2.33± 0.26%), 4.0 (2.92±0.03%), 4.5 (3.41±0.42%), 5.0 (4.14±0.12%), or 7.0 (3.81±0.05%), with no significant (P > 0.05) difference were observed between pH 5.0, 5.5, 6.0 and 6.5. While mean carbon content of DDG at pH 3.0 (16.73± 1.38%), 3.5 (12.97± 0.84%), 4.0

(11.57± 1.11%), 4.5 (10.60± 2.07%), 5.0 (8.57± 1.86%), 5.5 (7.48± 1.20%), 6.0 (8.28± 0.84%), 6.5 (7.34± 0.33%), or 7.0 (9.34± 0.69%).

Effect of incubation period

Incubation period significantly ($P<0.05$) affected ethanol yield (Fig 6A), nitrogen and carbon content of DDG (Fig 6B). Ethanol yield increased between 3 and 5 days, then stagnant from 6 to 9 days total incubation. Ethanol yield 2 (0.37 ± 0.11%) and 3 days (0.94± 0.05%) were significantly ($P<0.05$) lower than that at 4 days (4.06±0.46%), with both 4 days ethanol yield significantly ($P < 0.05$) lower than that at 5 (19.71± 0.38%), 6 (20.32± 0.42%), 7 (20.59± 0.33%), 8 (20.65± 0.48%), and 9 days (20.70± 0.46). Differences in ethanol yield between 5 and 6 days incubation were not significant ($P > 0.05$). Ethanol yield at 5 days was also significantly ($P < 0.05$) lower than 7, 8 or 9 days, but no significant ($P < 0.05$) difference between 6, 7, 8 and 9 days ethanol yields. Nitrogen content of DDG increased between 1 and 7 days, then relatively stagnant from 7 to 9 days total incubation. Nitrogen content of DDG 1 day (0.32± 0.05%) 2 (0.71± 0.11%), 3 (1.81± 0.10%), 4 (3.18± 0.08%), 5 (3.58± 0.25%), 6 days (4.40± 0.11%) were significantly ($P<0.05$) lower than that at 7 (4.67± 0.08%), 8 (4.47±0.07%) and 9 days (4.35±0.20%). Differences in nitrogen content of DDG between 1, 2, 3, 4, 5 and 6 days incubation were insignificant ($P < 0.05$), but no significant ($P > 0.05$) difference were observed between 7, 8 and 9 days incubation. While mean nitrogen content of DDG decreased between 1 and 4 days, then relatively stagnant from 5 to 9 days total incubation. Carbon content of DDG 1 day (46.57±1.57%), 2 (43.16± 2.68%), 3 (23.80± 0.26%), and 4 (12.90± 0.41%) were significantly ($P<0.05$) higher than that at 5 (9.72± 0.05%), 6 (9.20± 1.01%), 7 (8.68± 0.25%), 8 (8.30± 0.07%) and 9 days (7.29± 0.47%). Differences in nitrogen content of DDG between 1, 2, 3, and 4 days incubation were insignificant ($P < 0.05$), but no significant ($P > 0.05$) difference were observed between 5, 6, 7, 8 and 9 days incubation.

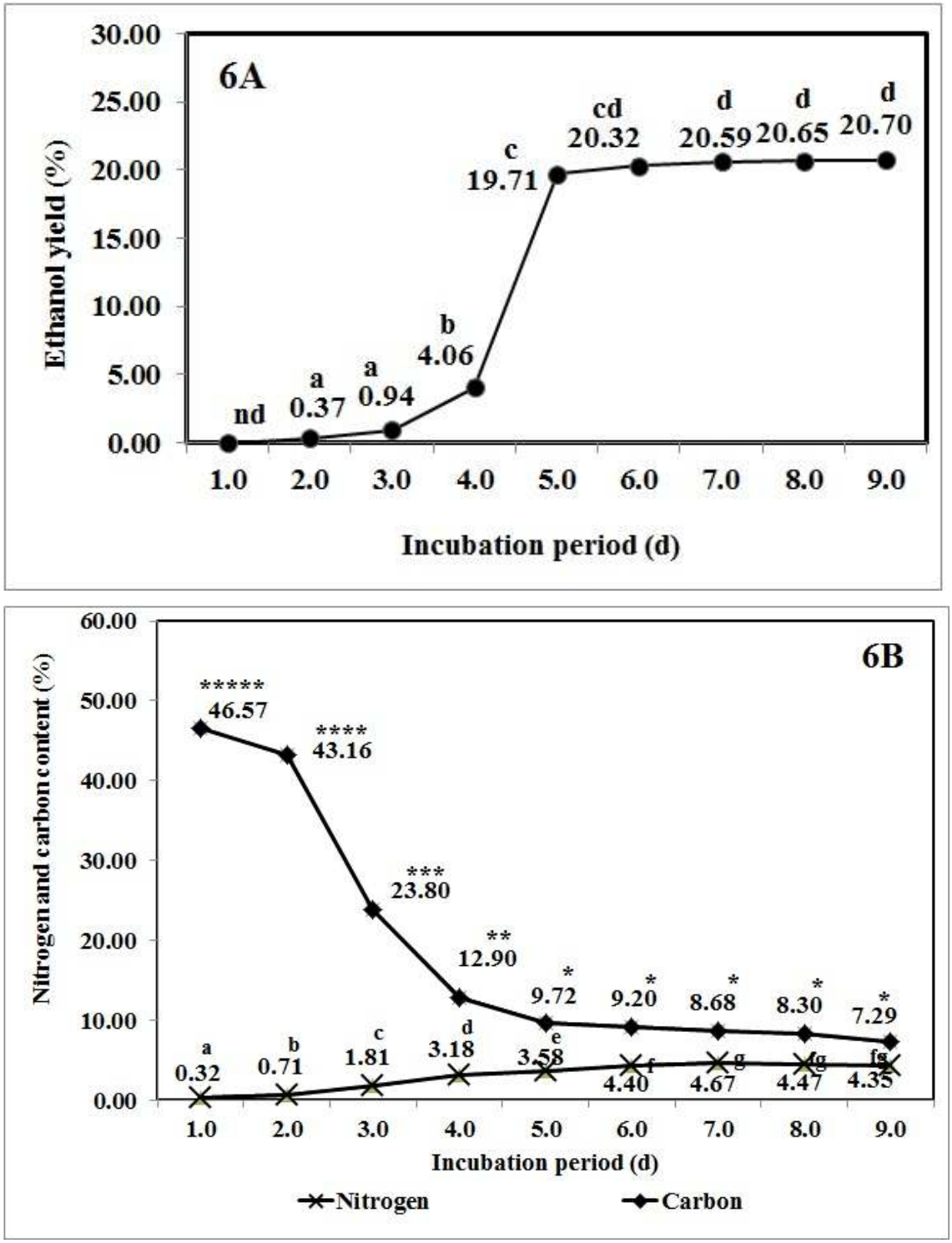


Figure 6 Effect of incubation period on ethanol yield (6A) and nitrogen and carbon content (6B) of distillate residue fermented rice husk hydrolysate medium by co-culture *S. cerevisiae* and *C. tropicalis*

DISCUSSION

Nitrogen deficiency in the fermentation medium will leads to slow and stuck fermentation rate (Vilanova et al., 2007). Nitrogen sources are very crucial and strongly influence the yeast growth and metabolism during fermentation (Beltran et al., 2005). In this work shows no significant different of ethanol yield or nitrogen and carbon content of DDG at exogenous nitrogen sources supplementation (NH_4NO_3, urea and $NaNO_3$) to rice husk fermentation media which indicated the co-culture *S. cerevisiae* and *C. tropicalis* can utilize on the various sources of nitrogen for growth and stimulate ethanol production. Some investigators have reported varying effects of exogenous nitrogen sources supplementation to the lignocellulosic fermentation media on ethanol production by yeast. These results of this work are similar to several studies reported that supplementation of the various sources of nitrogen to fermentation media does not significantly effect on the production of ethanol. Chu et al. (2012) reported supplementation of $(NH_4)_2SO_4$ and yeast extract to the SSF of pretreated corn stover with dilute H_2SO_4 did not bring any change on ethanol yield using thermotolerant strain *S. cerevisiae* DQ1. Akaracharanya et al. (2011) reported $(NH_4)_2SO_4$ supplementation to the hydrolysate of cassava pulp (a waste from cassava starch production) did not enhance ethanol production by the yeast. Fern´andez-L´opez et al. (2012) reported the addition of yeast extract, ammonium sulfate, urea, and their combination to medium of sugar rich molasses that obtained during the second step of crystallization did not improve ethanol productivity significantly. Wang et al. (2012) reported at the integrated ethanol-methane fermentation system, ammonium and other component in the effluent promoted yeast growth and fermentation rate but did not increase the yield of ethanol. However, the results of this work differ from several studies reported that the supplementation of various sources nitrogen to fermentation media effected on ethanol production. P´erez-Carrillo et al. (2011) and Harde et al. (2014) reported the supplementation of exogenous nitrogen sources such as yeast extract, malt extract, peptone, and $(NH_4)_2SO_4$ to the natural growing media enhanced ethanol production in *S. cerevisiae.*

Initial sugar concentrations in the growth media can influence the specific rate of yeast growth and ethanol production (Tesfaw and Assefa, 2014). There are varieties of

yeast, which are used to convert molasses into ethanol and CO_2, like *saccharomyces services and Klyureomyces marxianus* (Parkash, 2015). This work shows supplementation of molasses in the growth media significantly ($P<0.05$) increases ethanol yield and nitrogen and carbon content in the media fermentation. Production of ethanol from molasses-based media by co-culture fermentation has been reported. Eiadpum et al. (2012) reported immobilized co-culture *Kluyveromyces marxianus* and *Saccharomyces cerevisiae* can improve ethanol production from both sugarcane juice and blackstrap molasses when the operating temperature ranged between 33 °C and 45 °C and generated maximal ethanol concentrations of 81.4 and 77.3 g/l, respectively.

Carbohydrates and nitrogenous compounds are two major components affecting yeast performance in fermentation. A high of nitrogen sources significantly increased the efficiency of fermentation and yeast yield (Tyagi and Ghose, 1980). Increasing the nitrogen concentration in the fermentation medium can increase the rate of fermentation, decrease the duration and a lack of nitrogen triggers sluggish fermentations (Alexandre and Charpentier, 1998; Fleet and Heard, 1992; Varela et al., 2004). The ratio of nitrogen sources to carbon sources in the medium can influence on the yeast growth and metabolism of *Saccharomyces cerevisiae* (Larsson et al., 1993). Nitrogen deficiency with a high sugar transporter turnover rate, which resulted in a loss of sugar uptake capacity in the cells (Salmon, 1989 and Bisson, 1999). In this works, 4 formulation of rice husk hydrolysate media with different supplemented inorganic nitrogen and molasses significantly ($P<0.05$) influenced on ethanol yield, nitrogen and carbon content of DDG.

The specific rate of yeast growth and ethanol production were influenced by pH fermentation medium (Tesfaw and Assefa, 2014). In this work shows initial pH medium effected on ethanol yield and the content of nitrogen and carbon at DDG. A wide range of optimum pH (4.0–8.0) was reported for *S. cerevisiae* JZ1C isolated from rhizosphere of Jerusalem artichoke using inulin and Jerusalem artichoke tuber as substrate at 35°C (Hu et al., 2012). Optimum pH for *S. cerevisiae* BY4742 was in the range of 4.0–5.0, when the pH was lower than 4.0, the incubation period was prolonged though the ethanol concentration was not reduced significantly and when the pH was above 5.0, the concentration of ethanol diminished substantially (Lin et al., 2012). Some investigators

have been reported effect of incubation period on ethanol production from lignocellulosic medium by co-culture fermentation. Wright (1988) reported the maximum ethanol production of 4% (w/v) from the wheat straw medium after 48 h of incubation employing process of simultaneous saccharifi cation and fermentation using *T. reesei* cellulase and *Kluyveromyces fragilis*. Sharma (2000) reported maximum ethanol yield and fermentation efficiency of 0.397 gg^{-1} and 77.84 per cent, respectively after 36 h of incubation at 30°C using mixed culture of *S. cerevisiae* and *P. tannophilus*. Verma *et al*. (2000) reported maximum ethanol concentration of 24.8 g L^{-1} at 48 hours of incubation from starch medium in a single step process by co-culture of amylolytic yeasts and *S.cerevisiae*

CONCLUSIONS

Inorganic nitrogen and molasses supplementation can increase the production of ethanol from rice husk hydrolysate medium by co-culture *S. cerevisiae* and *C. tropicalis*. The best formulation mediums to obtain maximum production of ethanol with pH 5.5 and incubation period of 6 days is 1000 ml rice husk hydrolysate, 16.0 g/l urea, 12.0 g/l $NaNO_3$, 12.0 g/l NH_4NO_3, 1.0 g/l KH_2PO_4, 0.7 g/l $MgSO_4 \cdot 7H_2O$, 20 ml/l molasses.

Conflict of Interests

The authors have not declared any conflict of interest.

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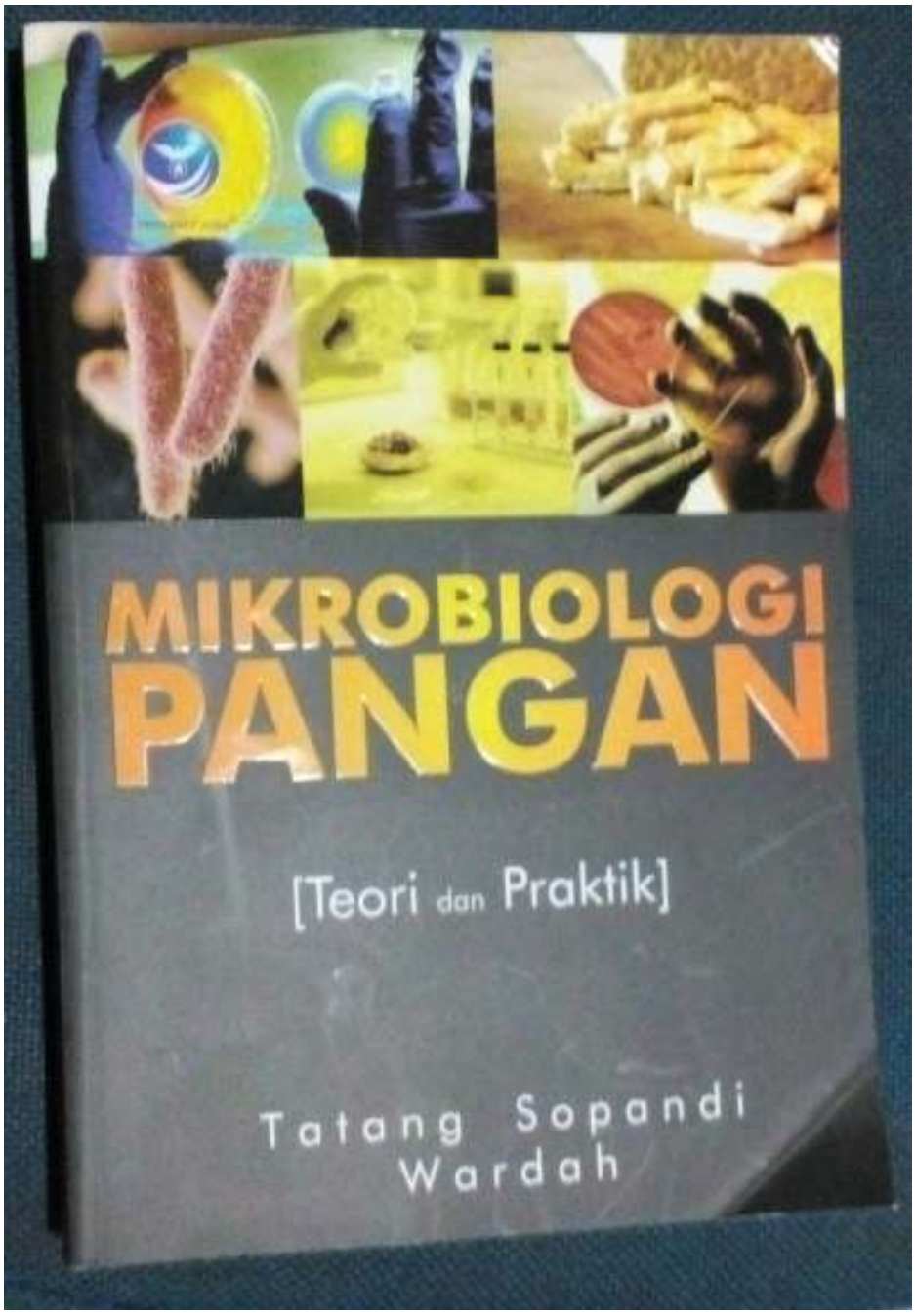
4. Buku ajar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PANGAN
[Teori dan Praktik]
Tatang Sopandi
Wardah

5.Buku ajar teknologi hasil pertanian

2. Seminar nasional

ANALISIS DATA PENELITIAN

1. Efek sumber nitrogen

Data hasil pengamatan efek penambahan sumber nitrogen terhadap kadar etanol serta kadar nitrogen dan karbon dalam DDG

Sumber Nitrogen	Ulangan	Kadar etanol (%)	Kadar Nitrogen DDG (%)	Kadar karbon DDG (%)
Urea	1	4.52	2.48	9.76
	2	5.54	2.3	11.41
	3	5.43	2.14	16.61
	4	4.96	1.79	14.35
	5	5.32	2.35	15.7
$NaNO_3$	1	4.67	2.70	9.25
	2	4.85	2.81	8.19
	3	4.93	2.36	10.82
	4	4.74	2.11	11.99
	5	5.53	2.05	12.38
NH_4NO_3	1	4.96	2.42	9.75
	2	4.86	2.55	8.84
	3	4.89	2.37	10.29
	4	4.76	2.56	14.18
	5	4.93	2.07	15.15

Analisis statistika efek sumber nitrogen terhadap kadar etanol

Descriptives

Etanol

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	5	5.1540	.41603	.18605	4.6374	5.6706	4.52	5.54
2	5	4.9440	.34246	.15315	4.5188	5.3692	4.67	5.53
3	5	4.8800	.07714	.03450	4.7842	4.9758	4.76	4.96
Total	15	4.9927	.31517	.08138	4.8181	5.1672	4.52	5.54

ANOVA

Etanol

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.205	2	.103	1.040	.383
Within Groups	1.185	12	.099		
Total	1.391	14			

Analisis statistika efek sumber nitrogen terhadap kadar nitrogen DDG

Descriptives

Nitrogen

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	5	2.2120	.26546	.11872	1.8824	2.5416	1.79	2.48
2	5	2.4060	.34136	.15266	1.9821	2.8299	2.05	2.81
3	5	2.3940	.19882	.08892	2.1471	2.6409	2.07	2.56
Total	15	2.3373	.27049	.06984	2.1875	2.4871	1.79	2.81

ANOVA

Nitrogen

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.118	2	.059	.783	.479
Within Groups	.906	12	.076		
Total	1.024	14			

Analisis statistika efek sumber nitrogen terhadap kadar karbon DDG

Descriptives

Karbon

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	5	13.5660	2.89692	1.29554	9.9690	17.1630	9.76	16.61
2	5	10.5260	1.78551	.79850	8.3090	12.7430	8.19	12.38
3	5	11.6420	2.82870	1.26504	8.1297	15.1543	8.84	15.15
Total	15	11.9113	2.69888	.69685	10.4167	13.4059	8.19	16.61

ANOVA

Karbon

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	23.648	2	11.824	1.811	.205
Within Groups	78.327	12	6.527		
Total	101.975	14			

2. Efek penambahan molases dalam media

Data hasil pengamatan efek penambahan molasses terhadap kadar etanol serta kadar nitrogen dan karbon dalam DDG

Penambahan molasses (ml/l)	Ulangan	Bobot kering DDG (g)	Kadar etanol (%)	Kadar nitrogen (%)	Kadar karbon (%)
0	1	4.45	5.47	2.40	8.34
0	2	4.87	4.91	2.59	7.75
0	3	4.62	5.34	2.62	6.68
0	4	4.57	5.55	2.66	8.38
0	5	4.67	5.28	2.56	8.34
5	1	5.15	7.45	2.77	8.93
5	2	4.91	6.07	2.80	9.02
5	3	4.96	6.67	2.81	9.56
5	4	5.48	7.09	2.88	10.61
5	5	5.26	7.03	2.93	10.69
10	1	5.27	8.69	3.00	11.92
10	2	6.08	8.67	3.01	11.66
10	3	5.66	8.76	3.05	11.39
10	4	5.92	8.70	3.11	10.40
10	5	5.36	8.01	3.11	10.95
15	1	5.92	9.47	3.28	14.70
15	2	6.34	9.69	3.32	12.17
15	3	6.25	9.60	3.36	13.59
15	4	6.84	9.00	3.94	13.40
15	5	6.22	9.68	3.35	12.56
20	1	7.63	9.91	3.66	15.71
20	2	7.42	9.58	3.78	12.23
20	3	7.53	9.80	3.72	13.51
20	4	7.38	9.84	3.78	15.21
20	5	7.07	9.64	3.65	12.75

Analisis statistika efek penambahan molasses terhadap bobot kering DDG

Descriptives

DDGS

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
0	5	4.6360	.15421	.06896	4.4445	4.8275	4.45	4.87
5	5	5.1520	.23167	.10361	4.8643	5.4397	4.91	5.48
10	5	5.6580	.34860	.15590	5.2252	6.0908	5.27	6.08
15	5	6.3140	.33374	.14925	5.8996	6.7284	5.92	6.84
20	5	7.4060	.21173	.09469	7.1431	7.6689	7.07	7.63
Total	25	5.8332	1.01212	.20242	5.4154	6.2510	4.45	7.63

ANOVA

DDGS

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	23.164	4	5.791	81.524	.000
Within Groups	1.421	20	.071		
Total	24.585	24			

DDGS

Tukey HSD

Molasses	N	Subset for alpha = 0.05				
		1	2	3	4	5
0	5	4.6360				
5	5		5.1520			
10	5			5.6580		
15	5				6.3140	
20	5					7.4060
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Analisis statistika efek penambahan molasses terhadap kadar etanol

Descriptives

etanol

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
0	5	5.3100	.24749	.11068	5.0027	5.6173	4.91	5.55
5	5	6.8620	.52203	.23346	6.2138	7.5102	6.07	7.45
10	5	8.5660	.31262	.13981	8.1778	8.9542	8.01	8.76
15	5	9.4880	.28665	.12820	9.1321	9.8439	9.00	9.69
20	5	9.7540	.13885	.06210	9.5816	9.9264	9.58	9.91
Total	25	7.9960	1.74215	.34843	7.2769	8.7151	4.91	9.91

ANOVA

etanol

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	70.710	4	17.678	165.847	.000
Within Groups	2.132	20	.107		
Total	72.842	24			

etanol

Tukey HSD

Molasses	N	Subset for alpha = 0.05			
		1	2	3	4
0	5	5.3100			
5	5		6.8620		
10	5			8.5660	
15	5				9.4880
20	5				9.7540
Sig.		1.000	1.000	1.000	.701

Means for groups in homogeneous subsets are displayed.

Analisis statistika efek penambahan molasses terhadap kadar nitrogen DDG

Descriptives

nitrogen

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
0	5	2.5660	.09990	.04468	2.4420	2.6900	2.40	2.66
5	5	2.8380	.06535	.02922	2.7569	2.9191	2.77	2.93
10	5	3.0560	.05273	.02358	2.9905	3.1215	3.00	3.11
15	5	3.4500	.27568	.12329	3.1077	3.7923	3.28	3.94
20	5	3.7180	.06261	.02800	3.6403	3.7957	3.65	3.78
Total	25	3.1256	.44122	.08824	2.9435	3.3077	2.40	3.94

ANOVA

nitrogen

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.284	4	1.071	55.240	.000
Within Groups	.388	20	.019		
Total	4.672	24			

nitrogen

Tukey HSD

Molasses	N	Subset for alpha = 0.05			
		1	2	3	4
0	5	2.5660			
5	5		2.8380		
10	5		3.0560		
15	5			3.4500	
20	5				3.7180
Sig.		1.000	.136	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Analisis statistika efek penambahan molasses terhadap kadar karbon DDG

Descriptives

karbon

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
0	5	7.8980	.72947	.32623	6.9922	8.8038	6.68	8.38
5	5	9.7620	.84615	.37841	8.7114	10.8126	8.93	10.69
10	5	11.2640	.60169	.26908	10.5169	12.0111	10.40	11.92
15	5	13.2840	.98455	.44030	12.0615	14.5065	12.17	14.70
20	5	13.8820	1.52103	.68022	11.9934	15.7706	12.23	15.71
Total	25	11.2180	2.43345	.48669	10.2135	12.2225	6.68	15.71

ANOVA

karbon

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	122.549	4	30.637	31.307	.000
Within Groups	19.572	20	.979		
Total	142.120	24			

karbon

Tukey HSD

Molasses	N	Subset for alpha = 0.05		
		1	2	3
0	5	7.8980		
5	5	9.7620	9.7620	
10	5		11.2640	
15	5			13.2840
20	5			13.8820
Sig.		.051	.156	.871

Means for groups in homogeneous subsets are displayed.

3. Efek formulasi media hidolisat sekam padi

Data hasil pengamanatan efek formulasi media hidrolisat sekam padi

Formulasi media	Ulangan	Kadar etanol (%)	Kadar nitrogen DDG (%)	Kadar karbon DDG (%)
F1 (1000 ml rice husk hydrolysate, 4.0 g/l urea, 3.0 g/l $NaNO_3$, 3.0 g/l NH_4NO_3, 1.0 g/l KH_2PO_4, 0.7 g/l $MgSO_4 \cdot 7H_2O$, 20 ml/l molasses) $MgSO_4 \cdot 7H_2O$, 20 ml/l molasses	1	16.44	2.78	12.56
	2	14.51	2.00	18.75
	3	14.09	2.72	13.68
	4	17.09	2.81	10.80
	5	17.03	2.74	14.43
F2 (1000 ml rice husk hydrolysate, 8.0 g/l urea, 6.0 g/l $NaNO_3$, 6.0 g/l NH_4NO_3, 1.0 g/l KH_2PO_4, 0.7 g/l $MgSO_4 \cdot 7H_2O$, 20 ml/l molasses)	1	19.05	3.36	9.65
	2	18.81	3.35	11.12
	3	18.42	3.17	13.08
	4	18.68	3.01	13.74
	5	18.80	3.29	11.44
F3 (1000 ml rice husk hydrolysate 12.0 g/l urea, 9.0 g/l $NaNO_3$, 9.0 g/l NH_4NO_3, 1.0 g/l KH_2PO_4, 0.7 g/l $MgSO_4 \cdot 7H_2O$, 20 ml/l molasses)	1	19.49	3.65	8.32
	2	19.64	3.60	9.71
	3	19.34	3.61	12.14
	4	19.77	3.14	8.58
	5	20.54	3.74	7.17
F4 (1000 ml rice husk hydrolysate, 16.0 g/l urea, 12.0 g/l $NaNO_3$, 12.0 g/l NH_4NO_3, 1.0 g/l KH_2PO_4, 0.7 g/l)	1	20.72	4.50	6.20
	2	19.83	3.79	8.97
	3	21.90	4.48	7.55
	4	19.74	3.95	6.29
	5	20.97	4.07	6.27

Analisis statistika efek formulasi media hidrolisat sekam padi terhadap kadar etanol

Descriptives

etanol

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	5	15.8320	1.42913	.63913	14.0575	17.6065	14.09	17.09
2	5	18.7520	.22906	.10244	18.4676	19.0364	18.42	19.05
3	5	19.7560	.46694	.20882	19.1762	20.3358	19.34	20.54
4	5	20.6320	.89004	.39804	19.5269	21.7371	19.74	21.90
Total	20	18.7430	2.02306	.45237	17.7962	19.6898	14.09	21.90

ANOVA

etanol

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	65.342	3	21.781	28.058	.000
Within Groups	12.420	16	.776		
Total	77.763	19			

etanol

Tukey HSD

Treat	N	Subset for alpha = 0.05		
		1	2	3
1	5	15.8320		
2	5		18.7520	
3	5		19.7560	19.7560
4	5			20.6320
Sig.		1.000	.308	.421

Means for groups in homogeneous subsets are displayed.

Analisis statistika efek formulasi media hidrolisat sekam padi terhadap kadar nitrogen DDG

Descriptives

nitrogen

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	5	2.6100	.34278	.15330	2.1844	3.0356	2.00	2.81
2	5	3.2360	.14724	.06585	3.0532	3.4188	3.01	3.36
3	5	3.5480	.23467	.10495	3.2566	3.8394	3.14	3.74
4	5	4.1580	.31901	.14267	3.7619	4.5541	3.79	4.50
Total	20	3.3880	.62486	.13972	3.0956	3.6804	2.00	4.50

ANOVA

nitrogen

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6.234	3	2.078	28.081	.000
Within Groups	1.184	16	.074		
Total	7.419	19			

nitrogen

Tukey HSD

Treat	N	Subset for alpha = 0.05		
		1	2	3
1	5	2.6100		
2	5		3.2360	
3	5		3.5480	
4	5			4.1580
Sig.		1.000	.303	1.000

Means for groups in homogeneous subsets are displayed.

Analisis statistika efek formulasi media hidrolisat sekam padi terhadap kadar karbon DDG

Descriptives

karbon

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	5	14.0440	2.96453	1.32578	10.3631	17.7249	10.80	18.75
2	5	11.8060	1.62913	.72857	9.7832	13.8288	9.65	13.74
3	5	9.1840	1.88296	.84208	6.8460	11.5220	7.17	12.14
4	5	7.0560	1.20879	.54059	5.5551	8.5569	6.20	8.97
Total	20	10.5225	3.28537	.73463	8.9849	12.0601	6.20	18.75

ANOVA

karbon

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	139.283	3	46.428	11.290	.000
Within Groups	65.797	16	4.112		
Total	205.080	19			

karbon

Tukey HSD

Treat	N	Subset for alpha = 0.05		
		1	2	3
4	5	7.0560		
3	5	9.1840	9.1840	
2	5		11.8060	11.8060
1	5			14.0440
Sig.		.376	.213	.334

Means for groups in homogeneous subsets are displayed.

4. Efek pH awal media hidolisat sekam padi

Data hasil pengamanatan efek pH awal media hidrolisat sekam padi

pH awal media	ulangan	Kadar etanol (%)	Kadar nitrogen DDG (%)	Kadar Karbon DDG (%)
3.0	1	1.78	1.56	16.26
	2	1.95	1.67	15.67
	3	1.24	1.93	16.23
	4	1.53	1.84	18.75
3.5	1	2.86	2.04	12.87
	2	2.38	2.26	13.65
	3	2.05	2.34	13.54
	4	2.17	2.67	11.82
4.0	1	8.52	2.96	11.24
	2	7.89	2.89	10.63
	3	8.32	2.92	11.23
	4	8.27	2.89	13.18
4.5	1	9.23	2.78	13.14
	2	9.56	3.54	11.21
	3	9.19	3.65	8.32
	4	8.93	3.67	9.71
5.0	1	12.69	4.12	10.56
	2	12.23	4.23	9.53
	3	14.15	4.24	7.85
	4	13.71	3.98	6.35
5.5	1	20.23	4.34	8.97
	2	20.82	4.26	7.87
	3	19.83	4.53	6.79
	4	21.18	4.65	6.27
6.0	1	19.68	4.56	9.058
	2	19.44	4.49	8.83
	3	19.79	4.35	7.98
	4	19.74	4.27	7.23
6.5	1	19.97	4.23	7.76
	2	18.72	4.39	7.39
	3	18.88	4.75	7.23
	4	18.83	4.18	6.98
7.0	1	12.49	3.84	9.74
	2	11.75	3.79	9.43
	3	11.53	3.74	9.85
	4	11.35	3.86	8.35

Analisis statistika efek pH awal media hidrolisat sekam padi terhadap kadar etanol

Descriptives

Etanol

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
3	4	1.6250	.30925	.15462	1.1329	2.1171	1.24	1.95
3.5	4	2.3650	.35707	.17854	1.7968	2.9332	2.05	2.86
4	4	8.2500	.26319	.13159	7.8312	8.6688	7.89	8.52
4.5	4	9.2275	.25851	.12925	8.8162	9.6388	8.93	9.56
5	4	13.1950	.88760	.44380	11.7826	14.6074	12.23	14.15
5.5	4	20.5150	.60158	.30079	19.5577	21.4723	19.83	21.18
6	4	19.6625	.15500	.07750	19.4159	19.9091	19.44	19.79
6.5	4	19.1000	.58384	.29192	18.1710	20.0290	18.72	19.97
7	4	11.7800	.50080	.25040	10.9831	12.5769	11.35	12.49
Total	36	11.7467	6.81464	1.13577	9.4409	14.0524	1.24	21.18

ANOVA

Etanol

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1619.004	8	202.376	857.261	.000
Within Groups	6.374	27	.236		
Total	1625.378	35			

Etanol

Tukey HSD

pH	N	Subset for alpha = 0.05					
		1	2	3	4	5	6
3	4	1.6250					
3.5	4	2.3650					
4	4		8.2500				
4.5	4		9.2275				
7	4			11.7800			
5	4				13.1950		
6.5	4					19.1000	
6	4					19.6625	19.6625
5.5	4						20.5150
Sig.		.461	.148	1.000	1.000	.777	.284

Means for groups in homogeneous subsets are displayed.

Analisis statistika efek pH awal media hidrolisat sekam padi terhadap kadar nitrogen DDG

Descriptives

nitrogen

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
3	4	1.7500	.16633	.08317	1.4853	2.0147	1.56	1.93
3.5	4	2.3275	.26120	.13060	1.9119	2.7431	2.04	2.67
4	4	2.9150	.03317	.01658	2.8622	2.9678	2.89	2.96
4.5	4	3.4100	.42387	.21194	2.7355	4.0845	2.78	3.67
5	4	4.1425	.12121	.06060	3.9496	4.3354	3.98	4.24
5.5	4	4.4450	.17748	.08874	4.1626	4.7274	4.26	4.65
6	4	4.4175	.13150	.06575	4.2083	4.6267	4.27	4.56
6.5	4	4.3875	.25773	.12887	3.9774	4.7976	4.18	4.75
7	4	3.8075	.05377	.02689	3.7219	3.8931	3.74	3.86
Total	36	3.5114	.96353	.16059	3.1854	3.8374	1.56	4.75

ANOVA

nitrogen

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	31.265	8	3.908	85.902	.000
Within Groups	1.228	27	.045		
Total	32.493	35			

nitrogen

Tukey HSD

pH	N	Subset for alpha = 0.05					
		1	2	3	4	5	6
3	4	1.7500					
3.5	4		2.3275				
4	4			2.9150			
4.5	4			3.4100	3.4100		
7	4				3.8075	3.8075	
5	4					4.1425	4.1425
6.5	4						4.3875
6	4						4.4175
5.5	4						4.4450
Sig.		1.000	1.000	.060	.218	.421	.553

Means for groups in homogeneous subsets are displayed.

Analisis statistika efek pH awal media hidrolisat sekam padi terhadap kadar karbon DDG

Descriptives

karbon

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
3	4	16.7275	1.37536	.68768	14.5390	18.9160	15.67	18.75
3.5	4	12.9700	.84060	.42030	11.6324	14.3076	11.82	13.65
4	4	11.5700	1.11059	.55529	9.8028	13.3372	10.63	13.18
4.5	4	10.5950	2.06673	1.03336	7.3064	13.8836	8.32	13.14
5	4	8.5725	1.85549	.92774	5.6200	11.5250	6.35	10.56
5.5	4	7.4750	1.19893	.59947	5.5672	9.3828	6.27	8.97
6	4	8.2745	.83669	.41835	6.9431	9.6059	7.23	9.06
6.5	4	7.3400	.32690	.16345	6.8198	7.8602	6.98	7.76
7	4	9.3425	.68515	.34257	8.2523	10.4327	8.35	9.85
Total	36	10.3186	3.12737	.52123	9.2604	11.3767	6.27	18.75

ANOVA

karbon

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	299.538	8	37.442	23.632	.000
Within Groups	42.779	27	1.584		
Total	342.316	35			

karbon

Tukey HSD

pH	N	Subset for alpha = 0.05				
		1	2	3	4	5
6.5	4	7.3400				
5.5	4	7.4750				
6	4	8.2745	8.2745			
5	4	8.5725	8.5725			
7	4	9.3425	9.3425	9.3425		
4.5	4		10.5950	10.5950	10.5950	
4	4			11.5700	11.5700	
3.5	4				12.9700	
3	4					16.7275
Sig.		.404	.230	.274	.206	1.000

Means for groups in homogeneous subsets are displayed.

5. Efek waktu inkubasi

Data hasil pengamatan efek waktu inkubasi

Waktu inkubasi (hari)	Ulangan	Kadar etanol (%)	Kadar Nitrogen DDG (%)	Kadar karbon DDG (%)
1	1	0	0.26	48.75
	2	0	0.32	46.61
	3	0	0.34	45.7
	4	0	0.37	45.21
2	1	0.43	0.67	39.15
	2	0.48	0.62	44.7
	3	0.24	0.68	44.43
	4	0.32	0.86	44.35
3	1	0.98	1.68	24.18
	2	0.869	1.93	23.74
	3	0.93	1.78	23.68
	4	0.98	1.83	23.59
4	1	3.43	3.11	13.4
	2	4.51	3.25	13.08
	3	4.09	3.24	12.56
	4	4.21	3.12	12.56
5	1	19.23	3.51	9.76
	2	20.15	3.64	9.75
	3	19.82	3.87	9.71
	4	19.65	3.28	9.65
6	1	20.23	4.36	9.56
	2	19.77	4.32	9.25
	3	20.54	4.35	9.02
	4	20.72	4.56	8.97
7	1	20.14	4.61	8.93
	2	20.93	4.72	8.84
	3	20.64	4.6	8.58
	4	20.65	4.73	8.38
8	1	20.25	4.37	8.34
	2	20.21	4.48	8.34
	3	21.07	4.5	8.32
	4	21.06	4.51	8.19
9	1	20.26	4.34	7.75
	2	20.34	4.07	7.55
	3	21.08	4.48	7.17
	4	21.12	4.5	6.68

Analisis statistika efek waktu inkubasi terhadap kadar etanol

Descriptives

etanol

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	4	.0000	.00000	.00000	.0000	.0000	.00	.00
2	4	.3675	.10813	.05406	.1954	.5396	.24	.48
3	4	.9398	.05273	.02636	.8558	1.0237	.87	.98
4	4	4.0600	.45563	.22782	3.3350	4.7850	3.43	4.51
5	4	19.7125	.38283	.19141	19.1033	20.3217	19.23	20.15
6	4	20.3150	.41589	.20795	19.6532	20.9768	19.77	20.72
7	4	20.5900	.32873	.16437	20.0669	21.1131	20.14	20.93
8	4	20.6475	.48238	.24119	19.8799	21.4151	20.21	21.07
9	4	20.7000	.46332	.23166	19.9628	21.4372	20.26	21.12
Total	36	11.9258	9.67081	1.61180	8.6537	15.1979	.00	21.12

ANOVA

etanol

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3270.070	8	408.759	3.353E3	.000
Within Groups	3.291	27	.122		
Total	3273.361	35			

etanol

Tukey HSD

waktu	N	Subset for alpha = 0.05				
		1	2	3	4	5
1	4	.0000				
2	4	.3675	.3675			
3	4		.9398			
4	4			4.0600		
5	4				19.7125	
6	4				20.3150	20.3150
7	4					20.5900
8	4					20.6475
9	4					20.7000
Sig.		.851	.367	1.000	.304	.817

Means for groups in homogeneous subsets are displayed.

Analisis statistika efek waktu inkubasi terhadap kadar nitrogen DDG

Descriptives

nitogen

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	4	.3225	.04646	.02323	.2486	.3964	.26	.37
2	4	.7075	.10500	.05250	.5404	.8746	.62	.86
3	4	1.8050	.10408	.05204	1.6394	1.9706	1.68	1.93
4	4	3.1800	.07528	.03764	3.0602	3.2998	3.11	3.25
5	4	3.5750	.24664	.12332	3.1825	3.9675	3.28	3.87
6	4	4.3975	.10966	.05483	4.2230	4.5720	4.32	4.56
7	4	4.6650	.06952	.03476	4.5544	4.7756	4.60	4.73
8	4	4.4650	.06455	.03227	4.3623	4.5677	4.37	4.51
9	4	4.3475	.19822	.09911	4.0321	4.6629	4.07	4.50
Total	36	3.0517	1.62100	.27017	2.5032	3.6001	.26	4.73

ANOVA

nitogen

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	91.515	8	11.439	682.572	.000
Within Groups	.452	27	.017		
Total	91.968	35			

nitogen

Tukey HSD

waktu	N	Subset for alpha = 0.05						
		1	2	3	4	5	6	7
1	4	.3225						
2	4		.7075					
3	4			1.8050				
4	4				3.1800			
5	4					3.5750		
9	4						4.3475	
6	4						4.3975	4.3975
8	4						4.4650	4.4650
7	4							4.6650
Sig.		1.000	1.000	1.000	1.000	1.000	.928	.127

Means for groups in homogeneous subsets are displayed.

Analisis statistika efek waktu inkubasi terhadap kadar karbon DDG

Descriptives

karbon

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	4	46.5675	1.56636	.78318	44.0751	49.0599	45.21	48.75
2	4	43.1575	2.67586	1.33793	38.8996	47.4154	39.15	44.70
3	4	23.7975	.26235	.13117	23.3801	24.2149	23.59	24.18
4	4	12.9000	.41376	.20688	12.2416	13.5584	12.56	13.40
5	4	9.7175	.04992	.02496	9.6381	9.7969	9.65	9.76
6	4	9.2000	.26920	.13460	8.7716	9.6284	8.97	9.56
7	4	8.6825	.25038	.12519	8.2841	9.0809	8.38	8.93
8	4	8.2975	.07228	.03614	8.1825	8.4125	8.19	8.34
9	4	7.2875	.47105	.23553	6.5379	8.0371	6.68	7.75
Total	36	18.8453	14.93455	2.48909	13.7922	23.8984	6.68	48.75

ANOVA

karbon

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7775.777	8	971.972	856.069	.000
Within Groups	30.656	27	1.135		
Total	7806.432	35			

karbon

Tukey HSD

waktu	N	Subset for alpha = 0.05				
		1	2	3	4	5
9	4	7.2875				
8	4	8.2975				
7	4	8.6825				
6	4	9.2000				
5	4	9.7175				
4	4		12.9000			
3	4			23.7975		
2	4				43.1575	
1	4					46.5675
Sig.		.068	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.